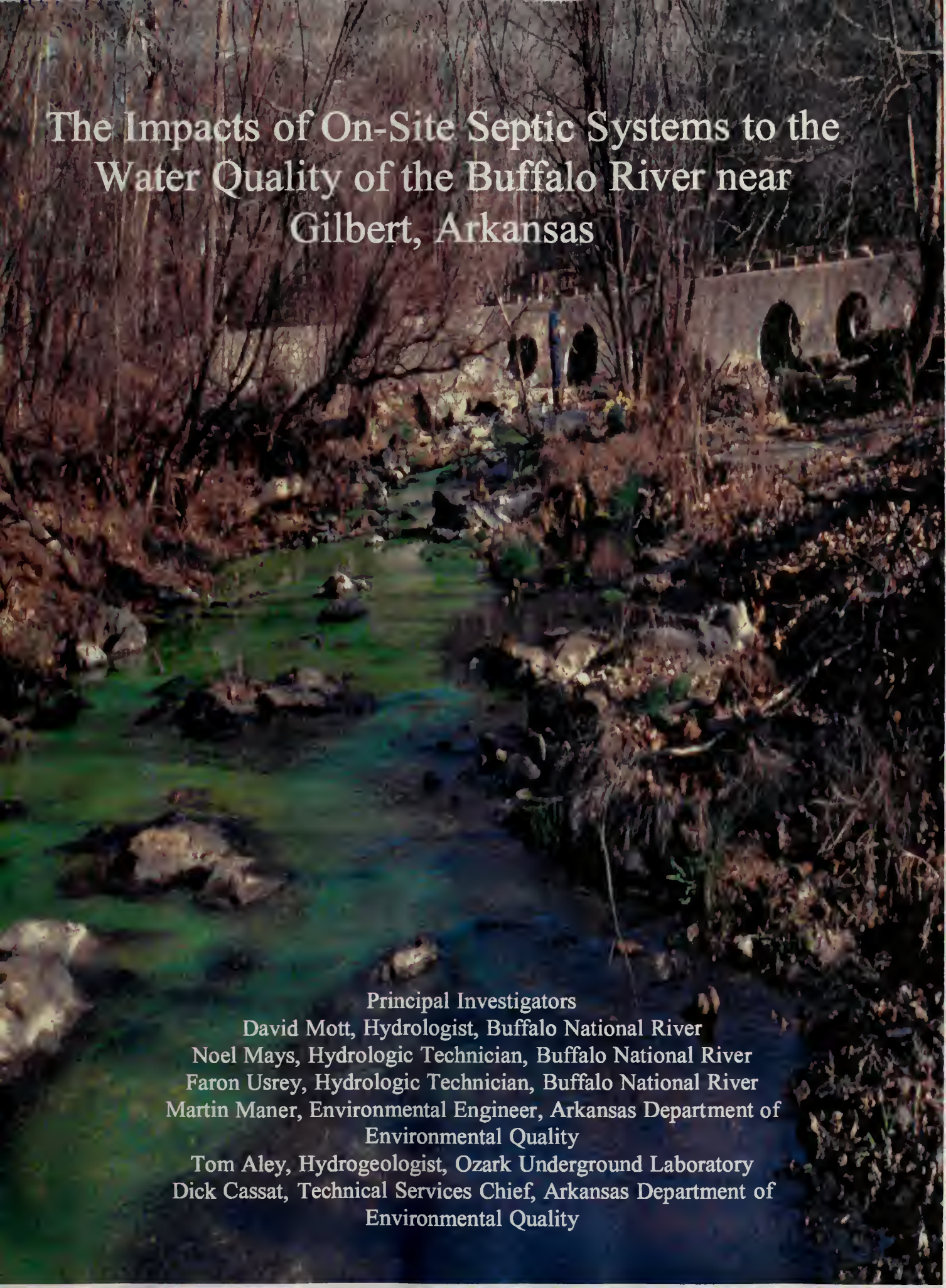
The Impacts of On-Site Septic Systems to the Water Quality of the Buffalo River near Gilbert, Arkansas



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Cover Photo:

Tom Aley injecting fluorescein dye into Dry Creek downstream of the Arkansas Highway 333 crossing during the time of travel trace.

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Executive Summary

The town of Gilbert's waste-water production is routed to individual on-site septic systems which are situated over karstic limestone bedrock within shallow soils. Karst systems are highly susceptible to groundwater pollution because of their internal drainage, numerous sinkholes, caves, and springs. Water quality monitoring over the past fifteen years at Gilbert Spring showed this spring to be the most contaminated spring within Buffalo National River. Fecal coliform bacteria counts have been measured far above the state standard of 200 col/100 mL, and average nitrate values are thirteen times higher than average nitrate values in the Buffalo River. Gilbert's ground water hydrology and septic systems were studied to quantify the impacts of Gilbert's septic fields on the water quality of Gilbert Spring and the Buffalo River. Should chronic failure of septic systems be documented, the need and feasibility of converting from on-site septic systems to a sewage treatment plant would also be investigated. The water quality studies and related assessments were conducted through a cooperative effort between Buffalo National River and the Arkansas Department of Environmental Quality (ADEQ).

A series of three town meeting were held at Gilbert to discuss this project with the mayor, city council members, and residents. The residents were initially concerned as to what they would be required to do if the investigators found problems. Buffalo National River's superintendent and representatives from ADEQ assured them that this was not an effort to force people into some type of compliance and that fair measures to correct problems would be sought. A number of residents volunteered to have their septic systems tested at this meeting. Over 30 individual dye traces were conducted from 29 septic systems in the town of Gilbert (85 percent of the targeted systems).

There were two major objectives defined for the septic system traces: 1.) determine if septic systems constructed in accordance with Arkansas State Regulations for septic systems are capable of functioning in the karst setting of Gilbert, and 2.) find any systems which are contributing leachate directly to Gilbert Spring. To address objective 1, we traced seven of the best septic systems identified in Gilbert simultaneously using two types of dye. Very small amounts of fluorescein were recovered from these traces, and we conclude that these systems are over 98 percent efficient in removing the tracer dye from the leachate. Our conclusion is that septic systems can and do work well in Gilbert's karst setting where they are properly constructed and maintained.

To address objective 2, the remaining 22 septic systems were traced using four types of tracer dyes over a three month period. Two systems were found to rapidly contribute dye directly to Gilbert Spring in large quantities. Two other systems contributed measurable but minor quantities of dye to Gilbert Spring, and the remaining 18 systems contributed negligible amounts of dye to Gilbert Spring. Of the two systems that contributed leachate directly to Gilbert Spring, one was found to be discharging to an old cistern, and the other was found to have a broken pipe running from the house to the septic tank. The system using the old cistern was completely replaced through cost-share assistance provided by Buffalo National River, and the system with the broken pipe was repaired by the landowner.

Previous dye tracing indicated a hydrologic connection between Dry Creek and Gilbert Spring. In an effort to better assess this connection, a time of travel study was conducted between Dry Creek and Gilbert Spring that showed a very open ground water conduit existed between the losing portion of Dry Creek and Gilbert Spring. Monthly or by-monthly flow measurements taken from Dry Creek and Gilbert Spring showed that often, Gilbert Spring's flow is completely accounted for by the flow lost from Dry Creek. During rain events, surface runoff is pirated by nearby solution valleys and contributes flow to Gilbert Spring. However, during base-flow conditions, Gilbert Spring is little more than the resurgence of Dry Creek.

The biologic community within Gilbert Spring's run (the stream formed below the spring) was assessed by comparing the macroinvertebrate (primarily aquatic insects) community from the run with the macroinvertebrate community in a nearby spring run formed below Mitch Hill Spring. The macroinvertebrate community was also assessed by performing various community metrics and comparing the results with results from other studies and information. These studies indicate that the Gilbert Spring run is a unique system with regard to its physical location in the Buffalo River floodplain and the frequent inundation with backwater when the river floods. In general, the aquatic community within Gilbert Spring's run was not significantly impacted by leachate from Gilbert's septic systems.

The majority of the water quality problems identified in Dry Creek and Gilbert Spring were relegated to nonpoint source pollution from agricultural operations in the Dry Creek basin. Dry Creek is currently listed as a targeted basin for implementation of Best Management Practices (BMPs) under a voluntary cost-share program administered by the Natural Resources Conservation Service (USDA, 1995). Landowners in the Dry Creek basin are eligible for 75% cost-share assistance to implement such BMPs as filter strips, waste storage structures, pasture and hay management, waste management systems, conservation easements, livestock exclusion and watering facilities, fencing, streambank protection, and critical area planting. A list of recommendations is provided at the end of the report.

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INTRODUCTION

The town of Gilbert, Arkansas, is the only town that adjoins Buffalo National River's boundary. Its incorporated limits lie less than 500 feet from the Buffalo River. The town's permanent population is only about seventy people, but summer tourism brings an additional 1,000 visitors to the town on a daily basis. Gilbert has one popular restaurant, numerous cabin rentals, bed and breakfast operations, canoe rentals, a general store, and other amenities. The town's waste-water production is routed to individual on-site septic systems which are situated over karstic limestone bedrock within shallow soils. Karst systems are highly susceptible to groundwater pollution because of their internal drainage, numerous sinkholes, caves, and springs. Several sinkholes lie within Gilbert and recharge through these sinkholes and nearby septic leach fields provides flow to one of the park's largest springs, Gilbert Spring (Appendix C, Figure C1 and C2), located between the town and the Buffalo River. Water quality monitoring over the past fifteen years has shown this spring to be the most contaminated spring within the park. Fecal coliform bacteria counts have been observed in excess of 17,000 col/100 mL (colonies per 100 milliliters) in Gilbert Spring (as compared to the state standard of 200 col/100 mL) and average nitrate values are thirteen times higher than average nitrate values in the Buffalo River. This project was initiated to: 1) conduct ground water and leachate tracing studies, 2) quantify the impacts of Gilbert's septic fields on the water quality of Gilbert Spring and the Buffalo River, 3) Assess biotic communities within Gilbert Spring and compare them to a reference spring and previous work, and 4) determine the need and feasibility of converting from on-site septic systems to a sewage treatment plant. The water quality studies and related assessments were conducted through a cooperative effort between Buffalo National River and the Arkansas Department of Environmental Quality (ADEQ).

STUDY AREA

Buffalo National River (BUFF) is a 150-mile long free-flowing stream in northern Arkansas famous for its scenic beauty as well as canoeing, fishing, and other recreational opportunities. The National Park Service's jurisdictional boundary includes a continuous 132-mile river corridor from near the headwaters to the confluence with the White River. The middle section of the Buffalo River, where Gilbert is located, has been designated by the Arkansas Department of Environmental Quality as impaired for water-based recreational use (Arkansas Department of Environmental Quality, 1992).

The town of Gilbert is situated within a massive sequence of soluble limestone. These formations have developed extensive karst drainage networks which are characterized by sinkholes, losing streams, caves and springs. The combination of thin soils and solutionally enlarged fractures which are common in karst landscapes often allows pollutants generated at the surface to migrate unfiltered into the underlying ground water system (White and White, 1989; Canter and Knox, 1986; D'Itri and Wolfson, 1987; Aley, 1982; Mott et. al., 1999). This is especially true with septic systems because subsurface leach lines installed in thin soils can intercept the bedrock/soil interface. Thus leachate

can be discharged directly into solutionally enlarged fractures and migrate rapidly into the subsurface drainage network or the water table.

BACKGROUND

In addition to being the America's first national river, the Buffalo River is designated an Extraordinary National Resource Water in Arkansas' water quality regulations. This standard contains an antidegradation clause which prohibits reduction of naturally occurring water quality. These standards also require fecal coliform counts to remain below 200 col/100 mL. Gilbert coincides with a river access point and this stretch of river is one of the most popular canoeing areas in mid to late summer.

In 1982, Aley sampled springs along the Buffalo River for the presence of optical brighteners which are found in laundry soaps and detergents. Aley concluded "the most seriously contaminated spring is Gilbert Spring...[which] is contaminated with sewage from the town of Gilbert." More recent monitoring by the National Park Service has similarly concluded that Gilbert Spring is the most contaminated spring in the park (Mott, 1997). Both elevated fecal coliform counts and nitrate concentrations are routinely observed at Gilbert Spring. Fecal coliform bacteria counts have been observed in excess of 17,000 col/100 mL and average nitrate values are thirteen times higher than average nitrate values in the Buffalo River.

Northwest Arkansas is one of the most rapidly growing areas in the nation. Gilbert's location near both the Buffalo River and a major interstate highway, has brought increasing populations and tourism. Numerous rental cabins and bed and breakfast operations, along with a popular restaurant, have recently opened. The transformation of this small town into a tourist attraction is occurring without any plan or assessment of the impacts of the increased sewage production on the resource tourists are coming to experience, Buffalo National River.

STUDY OBJECTIVES

Based on consultation between Buffalo National River and the Arkansas Department of Environmental Quality, the following objectives were developed. All locations discussed below are referred to in Appendix B1.

1) Conduct ground water and septic leachate tracing studies - Use various dyes (fluorescein, rhodamine WT, sulfarhodamine B, or eosine) to trace leaching sewage waste from septic fields, within the town of Gilbert. Previous work by Aley (Characterization of Groundwater Movement and Contamination Hazards on the Buffalo National River, Arkansas, Aley, 1982) had already confirmed the town of Gilbert to be in Gilbert Spring's recharge area. Activated charcoal dye receptors are recovered from Gilbert Spring frequently enough to allow estimation of travel times through the ground water system. Sample for optical brighteners to reconfirm the results of Aley's previous work. Systematically flush dyes into septic systems at various locations throughout Gilbert. The dye-detection network will include Gilbert Spring (and any other springs

within the area), surface streams, and the Buffalo River above and below the study area. The dye-tracing studies will be implemented immediately to allow potential adjustments to the water quality and biological monitoring as discussed below. Quantitative methodologies are used in the tracer studies.

2) Assess water quality impacts - Sample water quality from Gilbert Spring and the Buffalo River during base-flow conditions, and from Gilbert Spring during storm events (throughout the rising and falling portions of storm hydrographs). Base-flow sampling will determine the relative impact of Gilbert's septic leachate on Gilbert Spring, and the impacts of Gilbert Spring on the Buffalo River. Base-flow sampling will be performed on a bi-weekly basis April to September (tourist season), and on a monthly basis for the remainder of the year, over a two-year period. Storm-flow sampling will also be conducted. It will be important to collect both base-flow and storm-flow samples to determine if the septic field is acting as a point source (continuously supplying leachate and yielding higher pollutant concentrations during periods of low-dilution) or as a nonpoint source (contaminants flushed from leach fields by infiltrating rainfall). Impacts and relative contributions of pollutants to Gilbert will be assessed by sampling a losing stream (Dry Creek), which is the major recharge source for Gilbert Spring, at the point where it goes subsurface. This will allow us to quantify the relative amount of pollution coming from the Dry Creek watershed versus what we observe at the spring. Impacts to the Buffalo River will be assessed by sampling above and below the Gilbert Spring confluence. Parameters analyzed will include discharge, temperature, pH, dissolved oxygen, specific conductance, turbidity, fecal coliform bacteria, nitrate+nitrite nitrogen, soluble reactive phosphorus, ammonia, biochemical oxygen demand, chloride, and sulfate. Selected subsamples will be analyzed for metals, total Kjeldahl nitrogen, total phosphorus, total dissolved solids, total suspended solids, and chemical oxygen demand. Other parameters will also be analyzed as determined necessary based on more detailed field reconnaissance and allowed by ADEQ funding. All collection and analyses will use EPA, USGS, or *Standard Methods* protocols.

3) Assess biotic communities - Based on previous work and site conditions, we anticipate Gilbert Spring to be the predominant outlet for septic leachate. Even with the comprehensive water quality monitoring proposed, uncommon hazardous contaminants, or short-duration spikes of contamination could be missed. Because biological communities are exposed to the entire range of pollutants moving through a system, are always present, and give an indication of the effects of measurable pollutants on biotic communities, it will be important to perform community structure and function assessments. Buffalo National River already has an extensive database developed on macroinvertebrate community structure within springs, their diversity, abundance, and other matrices. As part of this study we will use quantitative techniques to collect benthic macroinvertebrate samples from both Gilbert Spring and a nearby reference spring, Mitch Hill Spring. We will collect four samples from each spring seasonally over the course of two years. We will identify specimens to the lowest taxonomic resolution possible as performed in the previously mentioned spring biological samples.

4) Need and feasibility assessment - Organize meetings with town representatives to gain their input, review the topography, land-ownership, and other aspects of the area, and determine the feasibility of converting to a sewage treatment plant. Personnel from ADEQ have the most experience in this arena, and can develop both preliminary plans and estimates of costs, if justified. The findings of the investigation will then be used to develop recommendations for future action. If these investigations reveal that Gilbert's septic systems are not a significant problem, other alternatives such as no action, or replacement of selected septic systems, may be recommended. Sources of funding for sewage treatment plant construction will be investigated if that is the preferred alternative.

METHODS

Water Quality

All water samples were collected and analyzed following U.S. EPA (Keith, 1992) and/or standard methods protocol (APHA, 1992). Base flow samples were collected and analyzed in a similar fashion as part of the National Park Service monitoring program for the Buffalo National River.

There were a number of field and laboratory parameters measured during this project.

1. rainfall
2. discharge
3. conductance
4. temperature
5. dissolved oxygen
6. pH
7. nitrate
8. total Kjeldahl nitrogen (TKN)
9. total phosphate
10. total suspended sediments
11. turbidity
12. fecal coliform
13. ammonia
14. orthophosphate
15. total organic carbon
16. chloride
17. sulfate

The first six parameters were determined in the field. Bacteria and turbidity analyses were conducted at the Buffalo River Water Quality Laboratory. The Arkansas Department of Environmental Quality Laboratory analyzed all of the other parameters. A brief description of these parameters and the analytical methods used is discussed below.

Water samples were collected in new plastic containers (milk jugs) for the analysis of all laboratory parameters except bacteria and turbidity. Bacteria and turbidity samples were collected in sterile whirlpak bags. Once the bag was in position for sample collection underwater, the air tight seal on the bag was broken and the bag filled with a sample. Then the bag was closed and brought to the surface and sealed. Samples were collected as close to the center of the stream as possible in areas of mixing (mid width and 0.6 of total depth). Care was taken to avoid contaminating the sample by touching inner portions of collection container and by collecting water upstream of the collector. Sample containers were labeled with collection time, location and the initials of the

collector with water insoluble ink prior to collection of a sample. All samples were analyzed within U.S. EPA specified holding times.

Field Parameters

Rainfall

Rainfall was measured by an automated tipping bucket rain gauge that records the amount of precipitation to the nearest 0.04 inches. Rainfall measurements were taken continuously throughout each precipitation event. The rain gauge sites were at Marshall and St. Joe.

Discharge

Discharge was measured using a Marsh-McBirney model 2000 flow meter. A cross section was set up on a run with a relatively uniform depth and flow. A metering depth rod was used to measure velocity at 60% depth. Flow rates were calculated using the conversion formula; $Q = \Sigma w_n * d_n * v_n$

W_n = mean width at a particular station

D_n = mean depth at a particular station

V_n = mean velocity at a particular station

Dissolved Oxygen

Dissolved oxygen (DO) measurements were made with an Orion 840 DO meter by placing the probe of the hand held unit into the top 6-12 in of the stream in well mixed riffle. The measurements were taken and values were recorded when the device displayed a constant reading for several seconds. The meter was standardized using an air calibration chamber prior to each collection event as prescribed in the operating manual. Dissolved oxygen is a measure of the concentration of oxygen in solution in a liquid. In natural waters, it is dependent on biochemical oxygen demand, chemical oxygen demand, rate of atmospheric reaeration, photosynthesis, respiration and water temperature (Mott, 1990). The DO minimum for streams with watersheds 10-100 mi^2 in the Ozark Highlands is 6 mg/L for the primary season (temperature less than 22^O C) and 5 mg/L for the critical season (temperature equal to or greater than 22^O C) (ADPC&E, 1995).

pH

pH is the negative logarithm to the base 10 of the hydrogen-ion concentration of a solution. An Orion 290A pH meter was used to measure this parameter at the time of sample collection. The pH meter was standardized with two pH buffer solutions (7 and 10) prior to each collection event and then checked regularly against a standard to ensure proper calibration. Most natural waters are buffered solutions which resist changes in pH (Chow, 1964). All three tributaries have a large amount of limestone exposed which keeps the run-off well buffered. Arkansas water quality standards for pH state that pH levels must not fluctuate more than 1.0 in a 24 hour period and may not be above 9.0 or below 6.0 (ADPC&E, 1995).

Specific Conductance and Temperature

The meter used for conductance and temperature measurements was an Orion 122 conductivity meter. The probe was placed directly in the upper 6-12 in of the stream for measurements Conductance is a measure of the electrical conductance of the water. Conductance is not only dependent on the concentration of ions present but is also dependent upon ionic charge and water temperature. Because conductance is temperature dependent, it is reported at a standard temperature of 25 degrees Celsius, i.e., specific conductance. The conductivity meter was standardized with appropriate solutions prior to each collection event, and is automatically temperature compensated and displays specific conductance directly.

Laboratory Analyses

Nitrate

The analytical method used for nitrate (NO_3) was the Hydrogen Reduction Method (4500-NO_3) (APHA, 1992) which also measured nitrite ($N0_2$). The holding time for nitrate analyses is 48 hours. For the purposes of this study $N0_3$ and $N0_2$ are reported as nitrate+nitrite as nitrogen (NO_3-N) because nitrite converts rapidly to nitrate and, thus, nitrite concentration is negligible in natural waters.

Nitrate (NO_3) is a soluble form of the nitrogen. The primary source of nitrate in water is the end product of the aerobic stabilization of substances containing organic nitrogen (Tchobanoglous and Schroeder, 1987). Stream ecosystems may benefit from limited amounts of nitrogen. However, excessive amounts can lead to prolific growth of aquatic plants. In streams, nitrate can be quickly returned to the organic nitrogen state by photosynthetic processes of plants. Agricultural watersheds are especially susceptible to excessive nitrogen input due to land-use. Pasture lands may receive nitrate from inorganic fertilizers and/or animal wastes.

Total Kjeldahl Nitrogen

Samples for total Kjeldahl nitrogen (TKN) analyses were digested in sulfuric acid, potassium sulfate, and copper sulfate and then analyzed by the Specific Ion Method (4500-N_{org} without removal of ammonia) (APHA, 1992). TKN is the sum of organic nitrogen and ammonia. Seven days is the maximum storage time for analysis of TKN.

Total Phosphorus (TP)

Phosphorus samples have a 48-hour holding time. Samples were collected in polyethylene bottles and later digested in persulfate which frees phosphates from any sediments; i.e., total phosphorus in the water sample. The 4500-P Ascorbic Acid analysis method was used as specified in Standard Methods (APHA,1992).

Total phosphorus is the measure of both organic and inorganic forms of phosphate (Reddy, 1980) in unfiltered water samples. Agricultural fertilizers and biological wastes and residues are relatively high in phosphorus--all of which are common in drainage basins with significant agricultural activity.The ADEQ states that total phosphorus concentration limits may not exist at levels that promote excessive algal growth. As a general guideline 0.1 mg/L is cited as the total phosphorus limit for streams.

Orthophosphate
Orthophosphate (PO_4-P) is a readily soluble phosphate that is common in natural waters. This parameter requires filtration prior to analyses. Orthophosphate samples have a 48 hour holding time. They were analyzed using 4500-P Ascorbic Acid Method (APHA, 1992). Orthophosphate is the form of phosphate that can be used directly by algae (Bowen, 1978).

Total Suspended Solids
Total Suspended Solids (TSS) are defined (APHA, 1992) as all materials too large to pass through a 2.0 micron pore-sized filter. A water sample was filtered and then the dry weight of the material on the filter was reported TSS (APHA, 1992). TSS is important in terms of the effect of the sediments on aquatic organisms, especially when the suspended sediments are deposited on fish eggs or change the environment for benthic organisms.

Turbidity
Turbidity detracts from the aesthetic qualities of a stream and is defined as the ability of suspended and colloidal materials to diminish the penetration of light (Chow, 1964). Turbidity samples have a 48 hour holding time and were analyzed with a HACH 2100A turbidimeter at the Buffalo River Water Quality Field Laboratory for grab samples but the storm samples were analyzed by the Arkansas Department of Environmental Quality laboratory. Turbidity is a measure of the colloids and suspended sediments present in a water sample. The regulations for turbidity are that "there shall not be a distinctly visible increase in the turbidity of receiving waters attributable to industrial, agricultural, other waste discharges or in-stream activities. Specifically, in no case shall any such...activity cause the turbidity values to exceed" 10 NTU for the Ozark Highlands (ADEQ, 1995).

Fecal Coliform
The "Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkansas" states that for "Primary Contact Waters - Between April 1 and September 30, the fecal coliform content shall not exceed a geometric mean of 200/100 mL nor shall more than 10 percent of the total samples during any 30-day period exceed 400/100 mL. During the remainder of the calendar year, these criteria may be exceeded, but at no time shall the fecal coliform content exceed the level necessary to support secondary contact recreation" (ADPC&E, 1995). The Secondary Contact regulation states that "...fecal coliform content shall not exceed a geometric mean of 1000/100 mL, nor equal or exceed 2000/100 mL in more than 10 percent of the samples taken in any 30-day period." For Extraordinary Resource Waters, "At no time shall the fecal coliform content exceed a geometric mean 200 colonies/100 milliliters in any size of watershed." (ADPC&E, 1995).

Standard methods allow 6 hours maximum holding time between collection and incubation (APHA, 1992). Due to the relatively short holding time it was necessary to perform fecal coliform analyses (Membrane Filtration Method—9222.D, APHA, 1992) at the Buffalo River Water Quality Laboratory.

Total Organic Carbon
The organic carbon in the sample was measured using Standard Methods 18th Ed. Method 5310 B. Combustion –Infrared. This method uses heat and oxygen to convert organic carbon to carbon dioxide which was measured by a nondispersive infrared analyzer. The samples were acidified and sparged before analysis to remove inorganic carbon. After sparging the sample contains only the nonpurgeable organic carbon fraction of total carbon. Natural waters normally contain very little purgeable organic carbon so the results were reported as Total Organic Carbon.

Biochemical Oxygen Demand
The biochemical oxygen demand analysis is used to determine the amount of oxygen required to biodegrade organic material in the sample. It is an empirical test where the results are based upon the use of standardized laboratory procedures. The method used was Standard Methods 18th Ed. Method 5210 B, 5-day BOD. The procedure consists of measuring the oxygen in a sample, incubating the sample at 20°C in the dark for 5 days, then measuring the remaining oxygen. The amount of oxygen consumed is the 5 day BOD.

Total Dissolved Solids
Dissolved solids are defined as the portion of solids that passes through a filter of 2.0μm. The method used was Standard Methods 18th Ed. Method 2540 C., Total Dissolved Solids Dried at 180°C. Waters high in dissolved solids are unsuitable for drinking water and for many industrial uses.

Bromide, Chloride, Fluoride, and Sulfate
The anions were analyzed using EPA Method 300.0 Ion Chromatography. Chloride, fluoride, and sulfate have national secondary drinking water contaminant levels.

Metals and Silica
The metals and silica were analyzed using EPA Methods 200.7 Inductively Coupled Plasma-Atomic Emission Spectrometry, and 200.8 7 Inductively Coupled Plasma-Mass Spectrometry. Dissolved metals have many effects in water. Some are beneficial and necessary for aquatic organisms others are very toxic.

Hardness
Hardness is defined as the sum of the calcium and magnesium concentrations, both expressed as calcium carbonate, in milligrams per liter. The hardness was determined using Standard Methods 18th Ed. Method 2340 B., Hardness by Calculation. The concentrations of calcium and magnesium were measured in the metals analysis.

Dye Tracing
Procedures for dye tracing are applicable to the following dyes employed in this study; Fluorescein, Rhodamine WT, Eosine, and Sulforhodamine B. Charcoal samplers are used to adsorb and concentrate dye and consist of fiberglass screening partially filled with 4.25 grams of activated coconut charcoal. Samplers (charcoal packets) are placed so as to be exposed to as much water as possible. In springs and streams they are typically attached to a rock or other anchor in a riffle area. Attachment of the packets utilizes

galvanized wire and packets extend outward from the anchor rather than being flat against it. Two or more separately anchored packets are typically used for sampling springs and streams.

Samplers are routinely collected and replaced from each of the sampling stations. The frequency of sample collection and replacement is determined by the nature of the study. Typically, the sampler collection frequency varies depending on the anticipated time of travel and concentration of dye in previous samplers. Common sample intervals are about one week, with initial collections being 1, 2, 4, and 7 days after the first dye injection.

Upon retrieval, the collected samplers are briefly rinsed in the water being sampled. The packets are shaken to remove excess water and placed in a plastic (Whirl-Pak) bag. The bag is labeled on the outside with a permanent type black marker pen to show the station name or number and the date and time of collection. Collected samplers are placed in the dark to minimize algal growth on the charcoal prior to the analysis work and are held and transported on ice. New charcoal samplers are routinely placed when used charcoal packs are collected. Routine sampler placement and recovery will be performed by the park hydrologist or hydrologic technician as directed and coordinated with the Ozark Underground Laboratory.

Each shipment of charcoal samplers must be accompanied by a sample tracking sheet which will also serve as the chain of custody form. Samples shipped to the Ozark Underground Laboratory are refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number. All samplers are logged in upon receipt and are recorded in a bound journal.

The standard elution solution is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide flakes to saturate the solution. The isopropyl alcohol is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This super-saturated layer is not used for elution. Preparation of eluting solutions uses dedicated glassware which is never used in contact with dyes or dye solutions.

Fifteen milliliters of the eluting solution is poured over the washed charcoal in a disposable sample beaker. The sample beaker is capped. The sample is allowed to stand for 60 minutes. After this time, the liquid is carefully poured off the charcoal into a new disposable beaker which has been appropriately labeled with the laboratory identification number. A few grains of charcoal may inadvertently pass into the second beaker; no attempt is made to remove these from the second sample beaker. After the pouring, a small amount of elutant will remain in the initial sample beaker. After the transfer of the elutant to the second sample beaker, the contents of the first sample beaker (the eluted charcoal) are discarded.

The Ozark Underground Laboratory uses both a Shimadzu RF-540 and a Shimadzu RF-5000U Spectrofluorophotometer capable of syncronous scanning. The RF5000U is the primary instrument used; the 540 is the back-up instrument.

A sample of the elutant is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 mL of the elutant is then placed in a disposable rectangular polystyrene cuvette. The cuvette has a maximum capacity of 3.5 mL. The cuvette is designed for fluorometric analysis; all four sides and the bottom are clear. The spectral range of the cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5000U or the RF-540. Both instruments are controlled by a programmable computer. Each instrument is capable of conducting substantial data analysis and are operated and maintained in accordance with the manufacturer's recommendations.

Typical analysis of an elutant sample includes synchronous scanning of excitation and emission spectra with a 17 nm separation between excitation and emission. The excitation scan is from 443 to 613 nm; the emission scan is from 460 to 630 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed is "very fast" and the typical sensitivity "high." The excitation slit for charcoal packet elutants is typically 5 nm, the emission slit is typically 2 nm on the RF-540 and 3 nm on the RF-5000U.

A plot of the synchronous scan for each sample is produced by the instrument; the plot shows emission fluorescence only. It is photocopied as a part of the final record. The synchronous scans are subjected to computer peak picks; peaks are picked to the nearest 0.1 nm. All samples run are stored on disk and printed on normal typing paper with a laser printer; sample information is also printed on the chart.

Dye quantities detected are presented in units of micrograms per liter (parts per billion). On the RF-540 the dye concentrations are calculated by separating fluorescence peaks due to the dyes from background fluorescence on the charts, and then measuring the height of the peak due to the dye. These heights are proportional to those obtained from standard solutions. On the RF-5000U the dye concentrations are calculated by separating fluorescence peaks due to dyes from background fluorescence on the charts, and then calculating the area within the fluorescence peak. This area is proportional to the area obtained from standard solutions. Where there are multiple fluorescence peaks, it is sometimes necessary to calculate dye concentrations based upon the height of the fluorescence peak rather than the area.

Dye concentration standards are run each day the machine is used. Six separate standards are used; the standard or standards appropriate for the analysis work being conducted is selected. All standards are based upon the as-sold weights of the dyes.

Laboratory blanks are run for every sample where the last two digits of the laboratory numbers are 00, 20, 40, 60, or 80. A charcoal packet is placed in a pumping well sampler and at least 25 gallons of unchlorinated water is passed through the sampler at a rate of about 2.5 gallons per minute. The sampler is then subjected tot he same analytical protocol as all other samplers. System functioning tests of the analytical instruments are conducted in accordance with the manufacturers recommendations

Ozark Underground Laboratory Quality Control/Quality Assurance procedures are attached in Appendix A.

SITE SELECTION

Water Quality Monitoring Sites

Water Quality monitoring sites were selected according to the objectives of the study. Sites were placed on Dry Creek, Gilbert Spring, Buffalo River downstream of the mouth of Gilbert Spring , and Buffalo River upstream of the mouth of Dry Creek (Appendix B1). The site on Dry creek was placed upstream of Arkansas Hwy 333 bridge this is approximately 200 meters upstream of the losing reach of the stream. The Dry creek site provided background water quality data before the water goes subsurface and passes under the town of Gilbert and resurfaces at Gilbert Spring. The water quality-monitoring site at Gilbert Spring was placed close to the mouth of the spring opening. Dry Creek data can be compared to data from Gilbert Spring to determine if Gilbert leachate is degrading water quality in Gilbert Spring. Similarly, the data from Gilbert Spring can be compared to the data from Dry Creek to determine the relative amount of pollution Dry Creek is carrying to Gilbert Spring.

The "above" water quality-monitoring site on the Buffalo River was placed on the first riffle upstream of the mouth of Dry Creek. This site was placed to give background data for water quality in the Buffalo River before it is influenced by Gilbert Spring. The "below" water quality-monitoring site on the Buffalo River was placed on the first riffle downstream of the mouth of Gilbert Spring Branch. Sampling below this rifle allowed for mixing of water from Gilbert Spring and Buffalo River to better determine if Gilbert Spring is negatively affecting the Buffalo River. Data from Buffalo River downstream mouth of Gilbert Spring can be compared to data from the site on the Buffalo River upstream the mouth of Dry Creek to directly determine the influence of Gilbert Spring on the Buffalo River. While the below sample site was 1200 feet below the confluence of Gilbert Spring, we realize that full mixing may not have occurred as of the first riffle. We will interpret the water quality data appropriately.

Dye Receptor Sites

Dye receptors were placed in consultation with Ozark Underground Laboratory hydrogeologist and Buffalo National River (BUFF) hydrologist. These sites were placed, according to field observations, in areas where dye reception was most probable. Factors that influenced the site selection were; background or control sites, site elevation, drainage area recharge, and probability of receiving dye. These factors are discussed in detail in the following sections. Four receptor sites were used throughout the study. These four sites were chosen after the time of travel trace was completed (a trace that

involved injecting dye up gradient from Gilbert and monitoring its discharge to the Buffalo River down gradient from Gilbert). Receptor sites were; Dry Creek upstream of the Hwy 333 bridge, Gilbert Spring, Buffalo River upstream of the mouth of Gilbert Spring, and Buffalo River downstream of the mouth of Gilbert Spring. (Appendix B1). Dry Creek upstream of the Hwy 333 bridge was chosen as a control site throughout the study. This sampler showed that no significant amount of dye was moving down Dry Creek. Gilbert Spring was the location where the majority of the dye was recovered in the time of travel trace. Buffalo River upstream of the mouth of Gilbert Spring was chosen as a routine site because a small amount of dye was recovered at this site during the time of travel trace. The receptor site on the Buffalo River downstream from the mouth of Gilbert Spring was placed to determine if dye was escaping from any features downstream of the mouth of Gilbert Spring

Town Meetings at Gilbert

Three town meetings were held at Gilbert to discuss this project with the residents. The first of these three meetings (September, 1999) was used to introduce the project to the town of Gilbert by BUFF hydrologist and BUFF superintendent. This introduction involved giving the townspeople a background of why the project was being conducted (i.e. monitoring over the past fifteen years showed Gilbert Spring to be the most contaminated spring within the park), what the project would involve, and how it would be conducted (dye tracing studies and water quality monitoring of Dry Creek and Gilbert Spring). The residents were concerned as to what they would be required to do if the investigators found problems. The superintendent assured them that this was not an effort to force people into some type of compliance and that fair measures to correct problems would be sought. A number of residents volunteered to have their septic systems tested at this meeting.

The second meeting (October, 1999) involved BUFF Hydrologist introducing the "players" in the study (Martin Maner, Arkansas Department of Environmental Quality and Tom Aley, Ozark Underground Laboratory). BUFF hydrologist stated he would be in charge of the dye traces from the NPS side and that he would be hiring a technician who would do the majority of water sample collection, recovering dye receptors, and would be the main person in the area for the next two years. The townspeople of Gilbert were assured that they would be kept informed of any pertinent data collected. The mayor of Gilbert supplied the NPS with a map of the town of Gilbert with each residence given an identification number. This map also gave a list of the property owners in Gilbert that were participating in the study (Appendix B2). The third meeting (September, 2000) was held to present the findings of the dye tracing study. In this meeting all information was presented to the townspeople of Gilbert. BUFF hydrologist reported that septic systems did work in Gilbert when they are installed and maintained according to state specifications and that no treatment plant was necessary. However, some septic systems were leaching into Gilbert Spring, and these systems needed to be corrected (as discussed in more detatil in the following section). The study was summarized by both BUFF hydrologist and Ozark Underground Laboratory hydrogeologist. This meeting was concluded with BUFF hydrologist stating that the NPS would like to fix the leaking systems with the owners cooperation.

Section I
Dye Trace Studies and Results

DYE TRACE STUDIES AND RESULTS

Background Screening for Possible Dye Interference

Field observations were made by BUFF hydrologist, Ozark Underground Laboratory hydrogeologist and BUFF hydrologic technician to establish background data monitoring sites. Background samplers were placed before any dye tracing was performed. The data collected at these sites was used to test if any type of fluorescent dye was present in the ground or surface water systems. Ten background sampling stations were established to collect data and were set in locations that were determined to be pertinent to the study. Four stations were set in Dry Creek, three stations were set in the Buffalo River, two stations in Gilbert Spring, and one station in a spring within close proximity to Dry creek (Appendix B1). Samplers were changed on a weekly basis for a total of three weeks. All of the background data collected at the ten monitoring stations showed no type of dye in the ground or surface water systems.

Time of Travel Study

After background screening detected no dye in the ground or surface water systems, the first dye trace performed was a time of travel study. This study was conducted to establish the length of time it would take dye to move from the losing reach of Dry Creek (Appendix C Figure C3), under the town of Gilbert, and resurface in Gilbert Spring, a distance of 1.1 miles. This trace also served to determine where to place dye receptors throughout the duration of the septic system traces. This was accomplished by injecting dye up gradient from Gilbert and monitoring its discharge to the Buffalo River down gradient from Gilbert. Six and one half ounces of fluorescein dye was used for the time of travel trace. The mixture consisted of approximately 75% dye and 25% diluent. The dye was introduced into the flow of Dry Creek immediately downstream of the Arkansas Highway 333 crossing of Dry Creek on February 24, 2000 at 0915 hours (Appendix C Figures C5 and C6). Water samples were collected on 15 minute intervals, and charcoal receptors were collected on 2 hour intervals. The first arrival of dye at Gilbert Spring was detected in a water sample collected on February 24, 2000 at 1930 hours (about 10 hours after injection). The first recovery of dye at this spring from a charcoal sampler was in the sampler in place for the period from 1800 to 2000 hours on February 24, 2000. The peak detected dye concentration in water samples was 205 ppb (Figure 1) at 2330 hours on February 24, 2000 (about 14 hours after injection). The dye concentration in water samples had decreased to less than 10 ppb by 0800 hours on February 25, 2000. Based upon water samples, dye concentrations of greater than 10 ppb persisted for less than 11.5 hours after first detection. The peak dye concentration detected in charcoal samplers was 12,700 ppb in sampler in place on February 24, 2000 from 2200 to 2400 hours.

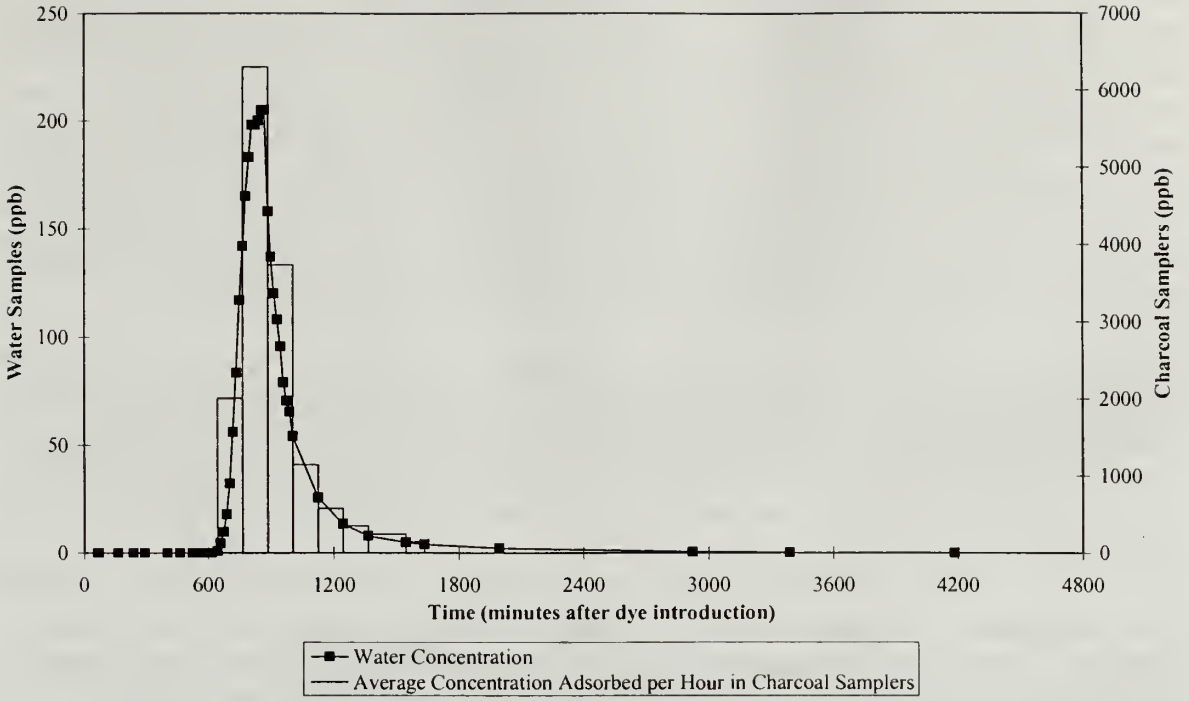

Figure 1: Dye breakthrough curve at Gilbert Spring resulting from Dry Creek time of travel study.

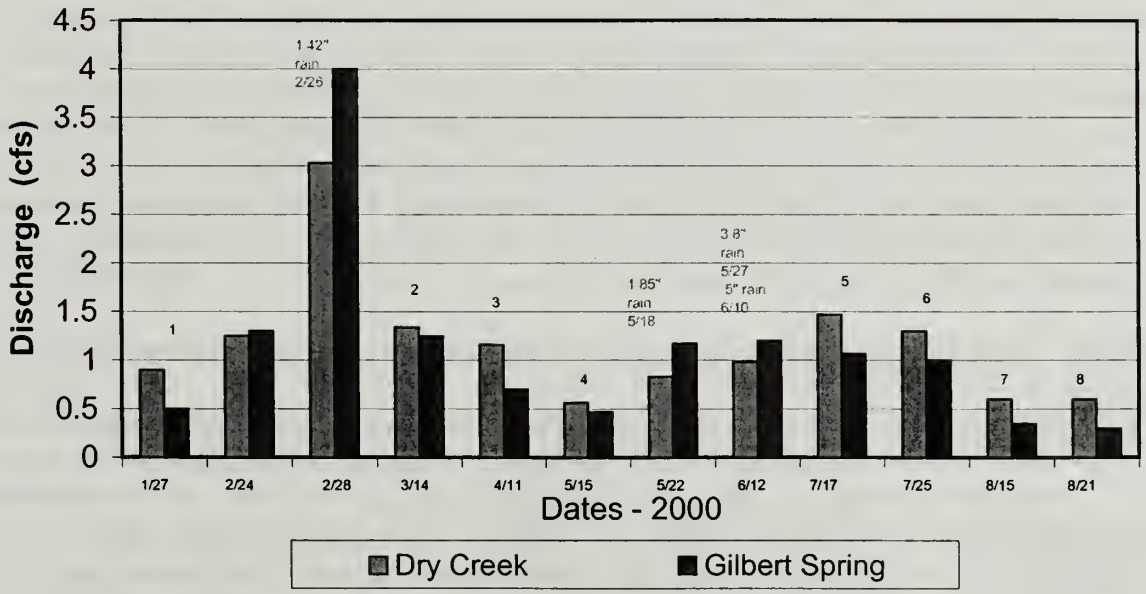

Figure 2: Discharge comparison of Dry Creek and Gilbert Spring.

Figure 2 shows the discharge measurements taken through August, 2000 at Dry Creek and Gilbert Spring. On 8 out of 12 measurements, Dry Creek had slightly more flow than Gilbert Spring. This pattern was observed during relatively dry conditions, it can be concluded that not all of the water sinking in Dry Creek emerges at Gilbert Spring. Seepage from the underground conduit between Dry Creek and Gilbert Spring was also observed in the time of travel study as indicated by a small amount of dye recovered from the site on the Buffalo River just upstream from Gilbert Spring (Table 3). During wet conditions, the flow of Gilbert Spring appears to exceed the flow of Dry Creek, indicating that Gilbert Spring receives recharge from other sources after rainfall periods.

Based on the time of travel study the Ozark Underground Laboratory hydrogeologist determined three weeks between dye injections would be adequate time for the system to flush before the same dye could be used again. The study also showed the underground portion of Dry Creek to be a very "open" system, and approximately 4.5 ounces of the 6.5 ounces of dye introduced was recovered at Gilbert Spring within 24 hours of injection.

This background information was used to design a sampling strategy for Gilbert's septic systems. We received permission to trace 29 of the 34 systems we desired to trace (about 85 percent of the systems more or less). Working with the consultant and ADEQ we honed our objectives and posed the following scenario "can septic systems work in the karst setting of Gilbert, or does the combination of soils, geology, and open ground water flow prohibit adequate renovation of septic leachate."

Strategy and Implementation of Septic System Traces

Septic systems in Gilbert were grouped into three categories in terms of their risk of causing groundwater contamination. The classes were High, Moderate, and Low Risk (Table 1). Dividing the septic systems in the town of Gilbert into these three categories assisted in the order and timing of dye injection at each site. Low risk systems were thought to be the least likely to contribute dye to Gilbert Spring, therefore these systems were traced first. The moderate risk systems were traced next. It was thought that moderate systems were a possible cause of contamination, but most likely these systems were not contributing to Gilbert Spring. High risk systems were traced last, these systems were thought to be the most likely avenue for contamination to Gilbert Spring.

Table 1: *Risk categories for Gilbert septic systems and the criteria used to evaluate each system*

Low Risk Systems	Systems less than ten years old which have been constructed in accordance with the State of Arkansas regulations and recommendations for septic systems.
Moderate Risk Systems	Systems which may not meet all current regulations and recommendations for new septic systems, but include at least a concrete tank and lateral lines.
High Risk Systems	Systems which are more likely to be failing based on age and lack of information about the septic system.

Charcoal packets were removed from Gilbert Spring on Mondays and Thursdays. Sampling consisted of collecting and replacing two charcoal receptors at each site. Both charcoal samplers were collected each time the station was visited. When charcoal samplers were collected both receptors were placed in a "whirl-pak" bag and refrigerated to keep samplers cool. Sampling was always conducted prior to making any dye introductions. Charcoal packets were mailed in coolers to Ozark Underground Laboratory (via "Priority Mail") along with the appropriate chain of custody forms. All dye mixtures were provided in a liquid form to facilitate ease of dye introduction. At least three weeks of sampling was conducted between the time a particular dye was introduced and the next time that dye was used for another septic system. Fluorescein and Rhodamine WT were introduced on the same dates. Eosine and Sulforhodamine B were introduced on the same dates. Using this approach we were able to conduct about 4 traces for every three week period until the initial round of dye tracing was completed. The standardized quantities of dye used are shown in Table 2.

Table 2: *Standardized quantities of each dye used in individual septic system traces.*

Fluorescein	3.5 oz.
Eosine	8.0 oz.
Rhodamine WT	16 oz.
Sulfarhodamine B	16 oz.

Nine of the septic systems in town were classed as Low Risk Systems. A standardized quantity of fluorescein dye was introduced into five of these systems (see photos Appendix C, Figures C7 and C8) on 3/16/00 before noon (i.e. 3.5 ounces of fluorescein was injected into each of the five systems for a total of 17.5 ounces of dye injected). Two moderate risk systems were traced on 3/16/00, one in the morning and one in the afternoon using the standardized quantity of rhodamine WT dye (see Table 2). These were the first dye introductions made in the septic system trace. We used a routine charcoal packet monitoring network as labeled in Appendix B1, which included four locations. The maximum dye recovery concentration (45.4 ppb fluorescein in charcoal) occurred within 6 days of the introduction. No rhodamine WT was detected from this round of tracing.

A fairly simple way of putting the dye recovery from the low risk system test into perspective is to compare maximum concentration recoveries from the low risk test to the time of travel study results. We introduced 290 percent more fluorescein into the septic systems and recovered only 1 percent as much dye at Gilbert Spring, relative to the direct introduction to the losing stream (Dry Creek). Mr. Aley with the Ozark Underground Laboratory estimates that these septic systems have a renovation efficiency in excess of 98 percent, as demonstrated by their ability to adsorb fluorescein (our surrogate dissolved contaminant and the most mobile of the dyes employed). Based on the low risk trace results, it is our contention that septic systems can and do work satisfactorily in the karst setting of Gilbert when properly installed.

After the initial low risk tracing, the septic systems were then divided into groups of four, these groups of four were then divided into pairs with one pair receiving one type of dye and the other pair receiving another type of dye, as discussed earlier in the strategy section. With this dye injection protocol, if any of the dyes appeared in Gilbert Spring at a given time, it could be associated with only the two systems that were injected with that type of dye. While the low-risk systems worked well throughout the study, two tests employing 4 moderate risk systems (systems 10, 24, 42, and 26) showed positive substantial dye recovery for Rhodamine WT as highlighted in red on Table 3. These four systems were re-traced to isolate which system(s) the dye was being contributed from (Appendix B Figure B2).

On 6/14/00 eosine was injected into system number 10 at 1733. There was no recovery at Gilbert spring from this trace. The next "re-trace" conducted was on the morning of 01/03/01, when Rhodamine W/T was injected into house number 26,eosine was injected into house number 42, and Fluorescein was injected into system number 24. Rhodamine W/T and Fluorescein were collected at Gilbert Spring, no eosine was detected. The septic systems at sites 26 and 24 were slated for repair because of the large amount of dye that they contributed to Gilbert Spring with concentrations in the 750 ppb range (Figure 3). The tracing also showed two additional systems leached detectable amounts of dye into Gilbert Spring, but these septic systems were not marked for repair due to the minimal amount of dye that they contributed to the spring. These are systems 34 and 22, and are also highlighted on Table 3. Their dye recovery concentrations were less than 20 ppb (Figure 3).

Figure 3: Dye Recoveries at Gilbert Spring

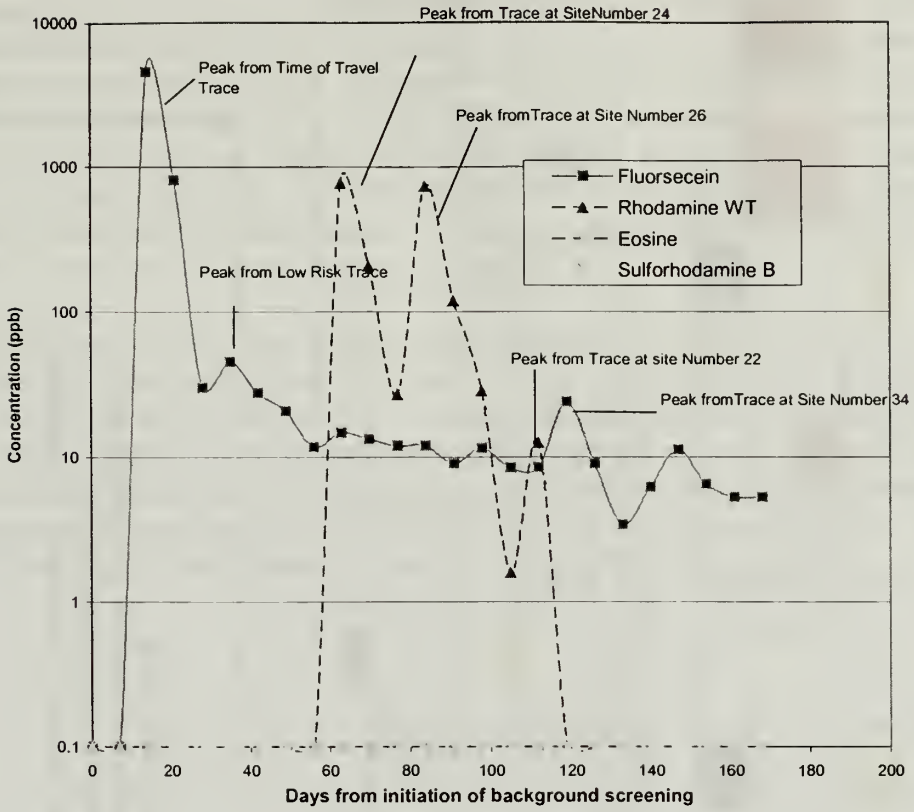

Table 3 provides a quantitative listing of how the study in Gilbert progressed. The four types of dye used are represented by the four colors associated with each type of dye, the calendar weeks that the dye trace study occurred are shown with the corresponding date above two columns labeled "in" and "out". The "in" column represents dye being injected into the ground or surface water system, the numbers in parenthesis show the house numbers into which the dye was injected (see Appendix B2 for spatial reference). The numbers in the brackets represent the weight [in ounces] of dye that was injected. The "out" column represents dye being collected at one of the sampling stations, the initials represent where the dye was collected (GS = Gilbert Spring). The numbers in parenthesis represent the concentration at which the dye was collected (in parts per billion).

Table 3: Quantitative printout of dye injection and recovery results.

Type of Dye	2/15 - 2/21		2/22 – 2/27		2/28 –3/6		3/7 – 3/13		3/14 – 3/20		3/21 – 3/27		3/28 – 4/3		4/4 – 4/10		4/11 - 417		4/18 – 4/24		4/25 – 5/1	
	In	out	In	Out	In	Out	In	Out	In	out	In	out	In	out	In	out	In	out	In	out	In	out
Fluorescein	Dry Creek [6 0z]	–	–	GS (4560) BRUS (2)	–	GS (807) BRUS (1.5)	–	GS (30) (5)	2 [3.5] 5 [3.5] 6 [3.5] 8 [3.5] 21[3.5]	GS (2.5) (45.4)	–	GS (27.5) (17.5)	–	GS (20) (20.7)	7[3.5] 36[3.5]	GS (11.7)		GS (14.7) (10.2) (13.8)	-	GS (6.8) (13.3)	9[3.5] 4[3.5]	GS (7.1) (11.9)
Eosine	–	–	–	–	–	–	–	-	–	–	43[8] 18[8]	–	–	–	–	–	30[8]	–	–	–	–	DC (.2)
Rhodamine_WT	–	–	–	–	–	–	–	–	3[16] 12[16]	–	–	–	–	–	10[16] 24[16]	–		GS (774) (301) (281)	–	GS (207) (159)	42[16] 26[16]	GS (26.2) (27)
Sulforhodemine_B	–	–	–	–	–	–	–	–	–	–	40[16] 44[16]	–	–	–	–	–	4[16] 2[16]	–	–	–	–	–

Type of Dye	5/2 – 5/8		5/9 – 5/15		5/16 – 5/22		5/23 - 5/29*		5/30 – 6/5		6/6 – 6/12		6/13 – 6/19*		6/20 – 6/26*		6/27 – 7/3		7/4 – 7/10		7/11 – 7/17	
	In	out	In	out	In	out	In	Out	In	out	In	out	In	out	In	out	In	out	In	out	In	out
Fluorescein	–	GS (6.2) (12)	–	GS (6.4) (9.0)	–	GS (10.5) (11.5)	–	GS (8.0) (8.4)	34[3.5]	GS (2.2) (8.5)	–	GS (11.4) (24.0)	–	GS (9) (4.7)	–	GS (3.4)	–	GS (5.8) (6.2)	27 [3.5]	GS (7.4) (11.2)	–	GS (3.2) (6.5)
Eosine	32[8]	–	–	–	–	–	–	–	–	–	–	–	10 [8]	–	–	–	–	–	–	–	–	BRUS (.1)
Rhodamine_WT	–	GS (44) (736)	–	GS (120) (71)	–	GS (90) (24.3)	–	GS (28.6) (20.7)	22[16]	GS (1.6) (1.4)	–	GS (3.2) (4.7) (12.6)	–	–	–	–	–	–	–	–	–	–
Sulforhodamine_B	32[16]	–	–	–	–	–	–	–	–	–	–	–	16[	–	–	–	–	–	–	–	–	–

Type of Dye	7/18 – 7/24		7/25 – 7/27	
	In	out	In	out
Fluorescein	–	GS (5.3)	–	GS (5.3) (3.0)
Eosine	–	–	–	–
Rhodamine_WT	–	–	–	–
Sulforhodamine_B	–	–	–	–

GS Gilbert Spring
DC= Dry Creek
BRUS= Buffalo River upstream Gilbert Spring
Numbers standing alone coincide with numbers on the associated map.
Numbers in Brackets = Amount of dye injected into systems.
Numbers in Parentheses = Concentrations of dye collected in charcoal packs.

* Storm events
5/26-5/27- 3.78 inches of rain
6/14-6/18- 3.08 inches of rain
6/21- 2.13 inches of rain

During one of the town meetings, Buffalo National River's Superintendent made the offer to buy all the materials to repair the leaking septic systems if the property owners agreed to pay all labor costs. The property owners at site 26 agreed with the cost-share program. The property owners did not have any knowledge of the history of their septic system, not even where it was located. The old "septic system" was located and was found to be, in actuality, an old cistern (see photos Appendix C Figures C9-C14). An elaborate new system was designed and installed according to Arkansas Department of Health standards. Buffalo National River purchased all supplies for the new septic system and the problem was corrected.

The septic system at site 24 was found to have a broken pipe between the house and the septic tank. When the septic system was uncovered, the septic tank was found to be empty and upon further exploration the broken pipe was found, the waste was being dumped directly into the ground. This property owner was not comfortable working with the National Park Service and did not participate in the cost-share program offered by the NPS. This information is based upon conversation with the property owners and other people in the town of Gilbert, none of the principle investigators were present during the repair of this septic system.

Section II
Biotic Community Assessment

Gilbert Spring Study
Macroinvertebrate Community Assessment
By
Faron D. Usrey
Buffalo National River

Introduction

The Buffalo National River (BUFF) has been collecting water-quality on spring systems for more than a decade, and subsequent analysis has shown that Gilbert Spring is highest in average nitrate concentration among the spring systems (Mott, 1997). The town of Gilbert, Arkansas is located above and adjacent to Gilbert Springs, and has no formal sewage treatment facilities. Disposal of raw, household sewage is through on-site septic systems. Since the town of Gilbert is located over a karst network, and is in close proximity to Gilbert Spring, septic leachate was suspected to be a factor in the high nutrient concentrations observed at Gilbert Spring.

In most aquatic systems, biological uptake and assimilation of nutrients into organisms is accomplished primarily by autotrophs such as aquatic macrophytes, phytoplankton, and epilithic periphyton. Assimilation rates of dissolved nutrients by periphyton are influenced by the availability of the nutrients and physical environmental parameters such as light, space, stream velocity, and water temperature. The aquatic community response to a moderate increase in nutrients typically includes an increase in periphytic density followed by shifts in macroinvertebrate community structure (Allan, 1996).

This section will investigate possible differences between the Gilbert Spring's macroinvertebrate community as compared to another system with lower nutrient concentrations, Mitch Hill Spring. Both spring systems have background information on water-quality and to some extent, the macroinvertebrate communities. Given the high nutrients at Gilbert Spring, the logical prediction is that differences in macroinvertebrate communities between the two communities could be explained by the excess nutrients at Gilbert Spring. The goals of this investigation are to determine if there are differences between the macroinvertebrate communities, and to provide possible explanations as to why the macroinvertebrate communities may differ in structure.

Methods

Seasonal variation within macroinvertebrate communities can be large (Barbour et. al., 1999); therefore, both hypocrenal (springbrook extending away from source) systems were sampled seasonally. Spring was considered between March through May, summer was June through August, fall was September through November, and winter was December through February. The discharges from the both spring systems were large compared to other systems within the Buffalo River watershed (Mott, 1997), and fluvial hydraulic habitats could be distinguished below the discharge sources. Of the hydraulic habitats observed, riffle habitats were chosen to represent the habitat for system comparisons. Riffle habitats are known to contain the greatest macroinvertebrate community diversity among fluvial geomorphic habitats. They are preferred as the habitat most suitable for macroinvertebrate community comparison studies (Plafkin et. al.,

1989, and Doisy and Rabeni, 1999). Relationships between macroinvertebrate taxa and their respective physical habitats are important in determining differences between sites and quantitative sampling at points within the riffle habitat preserves, as closely as possible, macroinvertebrate taxa dependencies upon physical habitat parameters. For this reason, a quantitative Hess sampler was used to preserve the faunal dependence upon the physical habitat gradients.

Hess sampling was conducted at three locations within each of the first three riffle habitats below the springs. The riffle habitat was divided into ten equidistal length and width axis points. The ten points were numbered 1 through 10, and a random selection was performed for both length and width axis producing 1 sampling point from within the riffle habitat. This was done 3 times for each of the 3 riffles sampled. This resulted in a sampling size of 9 per spring system per season, and a total of 36 samples per site, per year. Once the sample point was selected, the site was marked with an anchored buoy; each buoy was marked with the riffle number and sample number. The riffle closest to the source was labeled riffle number 1, and the top most sampling point was sample 1(i.e. R1-S1 represents the sample closest to the source). At these buoys, water depth, water velocity, and canopy density was measured. Care was taken to stay below all sample locations during the initial point collections. Once these measurements were taken, the sampler was placed into the substrate to the depth of approximately 10-cm. Prior to the active organism collection, 40 substrate particles were randomly selected, washed into the collection net and measured using calipers. The 40 particles were used to produce substrate size means and composition diversities. Once the interior substrates were measured, the remaining contents were stirred for 3 minutes and the suspended organisms were washed into the collection bucket. Samples were placed into containers with a 70% Ethanol preservative. Sample containers were labeled inside and out with site, date, riffle and sample number.

Sample processing was conducted within the laboratory. Macroinvertebrate samples were individually placed into a white picking pan that was divided into 10 sections. The sample was homogenized and organisms within a randomly chosen section were removed. Due to the large number of organisms within each sample, subsampling was conducted. In order to determine the number of organisms needed to represent a sample, three samples from each of the systems were processed in allocates of 100 organisms until the entire sample was processed. Adequate levels of organism counts were determined, and organisms collected from the subsampling were grouped by taxa and identified to the lowest practical taxonomic level. The genus level was achieved for most taxa groups. Numerous national and regional keys were used in identifying the macroinvertebrate taxa (Bednarik and McCafferty 1979, Kondratieff and Voshell 1984, Merritt and Cummins 1996, Pennak 1989, Pflieger 1994, Poulton and Stewart 1991, Provonsha 1990, Stewart and Stark 1993, and Wiggins 1998).

The macroinvertebrate community was characterized using taxa richness, Simpson's index of diversity, percent Ephemeroptera/Plecoptera/Trichoptera (EPT), percent Diptera, and a multimetric approach especially designed for the macroinvertebrate communities of the Buffalo River watershed (Mathis, 2001). Individual metrics was used to evaluate the community's responses to various gradients in physical habitat and water-quality. Taxa richness, Simpson's diversity, and percent EPT are expected to be reduced by a general increase in perturbation, and percent Diptera is expected to increase with increasing perturbation (Barbour et. al., 1999). An

Index of Community Integrity (ICI) was also used to evaluate the overall conditions of the macroinvertebrate communities. The ICI uses 10 common indices, which are known to react to perturbation in predicable a manner. The following indices are used in the generation of the ICI score: Margalef's Index of Taxa Richness, Shannon's Taxa Diversity Index, Percent Dominant Taxa, Percent Chironomidae, Percent Plecoptera, Percent Trichoptera, Percent Elmidae, Percent *Corbicula*, Percent Intolerant, and Percent Collector-Filterer. By adding together all the normalized scores for the metrics calculated on each site, a total ICI score was generated.

Water-quality measurements used in comparing two spring systems were taken from the NPS-BUFF water-quality database. A total of 138 records from 1990 until the fall of 2000 were divided into seasonal categories, and seasonal means were used in correlations with macroinvertebrate community metrics. Nitrates (NO_3) and orthophosphates (PO_4) were the nutrients of interest, and were selected to use in site comparisons.

Two-way Analysis of Variance (ANOVA) was used to determine differences between the two spring systems for the macroinvertebrate communities. The dependent variables for the macroinvertebrate communities were the community metrics previously mentioned. Two-way ANOVA was selected over a T-test because two effect categories (factors) were required to compare the spring communities, and possible interactions between categories were expected (site, season, and site*season). Potential associations between physical habitat and macroinvertebrate community metrics were generated using a Pearson's correlation matrix with a Bonferroni's post hoc test for significance, which accounts for pair-wise alpha inflation. Potential relationships between macroinvertebrate community indices and water-quality parameters were tested using a Spearman's rank correlation; however, with the small sample size a perfect correlative value was required before achieving significance. All assumptions for ANOVA, Pearson's, and Spearman's correlations were checked before the analyses were performed (Berk 1994, and Sokal and Rolf 1995).

Graphs that represented the variation within and among habitats and sites used box plots that are different from traditional box plots (Figure 1). The length of each box shows the range within which the central 50% of values fall, with the box hinges (borders) at the first and third quantiles. The whiskers show the range of values that fall within the inner fences (but do not necessarily extend all the way to the inner fences). Values between the inner and outer fences are plotted with asterisks, and values outside the outer fence are plotted with empty circles (Systat, Version 8).

Explanation of the Box Plot Distributions

Box Plots divide the data into four equal parts. The three borders separating the four parts are called the first, second, and third quartiles. ¼ of the data are to the left of the first quartile, ½ are to the left of the second quartile, and ¾ are to the left of the third quartile. The 2nd quartile is also the median. The length of each box shows the range within which the central 50% of the values fall.

Whiskers are the lines extending out from the main box. These indicate the upper and lower ranges of the data, but the whisker lengths are limited to no more than 50% of the box length. Whiskers also show the range of values that fall within the inner fences.

Hinges are values that fall at the beginning and end of the box length, the end of the 1st and 3rd quartile.

Inner Fences are the values that falls outside of the adjacental hinge.

Outer Fences are the values that fall at the extreme of the whisker, constrained by the 50% box length limit.

Lower inner fence = lower hinge (median lower hinge)
Upper inner fence = upper hinge + (1.5 (upper hinge median))
Lower outer fence = lower hinge (3 (median lower hinge))
Upper outer fence = upper hinge + (3 (upper hinge median))

Asterisks are plotted when value fall between the inner and outer fences.

Empty Circles indicate values outside the outer fences.

Figure 1. Explanation of Box Plot distributions by quartiles, whiskers, and outside values as presented within this investigation. Box Plots generally show within site variation for between site visual comparisons.

Results

A total of 12,830 macroinvertebrates were processed, sorted and identified resulting in 4 Phylum, 4 Classes, 10 Orders, 28 Families, and 33 individual taxa (Table 1). Mean macroinvertebrate taxa richness was highest at Gilbert Spring throughout all the seasons (Figure 2, Table 2). Mean macroinvertebrate diversity (Simpson's Index) was higher at Mitch Hill Spring throughout the seasons (Figure 3, Table 2). During spring, fall, and winter, Gilbert Spring exhibited higher % EPT than did Mitch Hill Spring (Figure 4, Table 2). Gilbert Spring had higher percentages of *Cheumatopsyche* and *Agapetus*; two members of the order Trichoptera than did Mitch Hill Spring. Mean % Diptera was also higher in Mitch Hill Spring for all seasons (Figure 5, Table 2). *Gammarus minus* (a.k.a. sideswimmer or scud) was the dominant organism in both of the hypocrenal systems. Although, Mitch Hill Spring had approximately 1.5 times more *Gammarus minus* than Gilbert Springs. ICI seasonal averages were consistently higher at Mitch Hill Spring for all seasons (Figure 8, Table 2).

Table 1. Taxonomic list of macroinvertebrates collected from Mitch Hill Spring and Gilbert Spring.

Phylum	Class	Order	Family	Genus and species
Annelida	Oligochaeta			
Arthopoda	Crustacea	Amphipoda	Gammaridae	Gammarus minus
Arthopoda	Crustacea	Isopoda	Asellidae	Lirceus
Arthopoda	Insecta	Coleoptera	Gyrinidae	Dineutus
Arthopoda	Insecta	Coleoptera	Elmidae	Optioservus
Arthopoda	Insecta	Coleoptera	Elmidae	Psphenus
Arthopoda	Insecta	Crustacea	Cambaridae	Orconectes
Arthopoda	Insecta	Diptera	Chironomidae	
Arthopoda	Insecta	Diptera	Empididae	Hemerodromia
Arthopoda	Insecta	Diptera	Elmidae	Ordobrevia
Arthopoda	Insecta	Diptera	Simullidae	
Arthopoda	Insecta	Diptera	Tipulidae	Tipula
Arthopoda	Insecta	Ephemeroptera	Beatidae	Beatis
Arthopoda	Insecta	Ephemeroptera	Isonychiidae	Isonychia bicolor
Arthopoda	Insecta	Ephemeroptera	Lepidostomatidae	Lepidostoma
Arthopoda	Insecta	Ephemeroptera	Leptophlebiidae	Leptophlebia
Arthopoda	Insecta	Ephemeroptera	Heptageniidae	Stenacron
Arthopoda	Insecta	Ephemeroptera	Heptageniidae	Stenonema femoratum
Arthopoda	Insecta	Meloptera	Chauliodinae	Nigronia
Arthopoda	Insecta	Plecoptera	Agrionidae	Calopteryx
Arthopoda	Insecta	Plecoptera	Uenoidae	Neophylax fuscus
Arthopoda	Insecta	Plecoptera	Perlidae	Perlesta
Arthopoda	Insecta	Plecoptera		
Arthopoda	Insecta	Trichoptera	Glossosomatidae	Agapetus
Arthopoda	Insecta	Trichoptera	Hydropsychidae	Cheumatopsyche
Arthopoda	Insecta	Trichoptera	Philopotamidae	Chimarra
Arthopoda	Insecta	Trichoptera	Philopotamidae	Dolophilodes
Arthopoda	Insecta	Trichoptera	Helicopsychidae	Helicopsyche
Arthopoda	Insecta	Trichoptera	Hydropsychidae	Hydropsyche scalaris
Arthopoda	Insecta	Trichoptera	Psychomyiidae	Psychomyia
Arthopoda	Insecta	Trichoptera	Limnephilidae	Pycnopsyche
Arthopoda	Insecta	Trichoptera	Hydroptilidae	Stacobiella
Mollusca	Bivalvia	Pelecypoda	Corbiculidae	Corbicula fluminea
Mollusca	Gastropoda			
Nematomorpha				
Tubellaria				

Table 2. Seasonal means of macroinvertebrate community metrics from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Substrate Diversity	Substrate Average	Taxa Richness	Taxa Diversity	% EPT	%Diptera	%Gammarus	%Agapetus	ICI
S33	Spring	0.381	36.3	5.4	0.535	6.8	9.3	66.8	6.2	44
S33	Summer	0.371	41.8	6.1	0.523	13.6	17.4	66.3	0.7	45.3
S33	Fall	0.417	38.4	5.2	0.756	6.8	4.2	86.4	1.3	44.7
S33	Winter	0.388	36.5	7.1	0.604	13.1	6.9	76.1	4.0	44.7
S41	Spring	0.444	28.8	9.1	0.350	12.9	2.4	49.1	6.8	35.3
S41	Summer	0.393	18.4	6.9	0.404	6.4	1.0	40.5	5.0	40.7
S41	Fall	0.410	24.0	8.9	0.330	43.6	2.4	43.5	14.9	38
S41	Winter	0.499	18.1	8.6	0.379	18.8	1.7	53.9	11.6	42

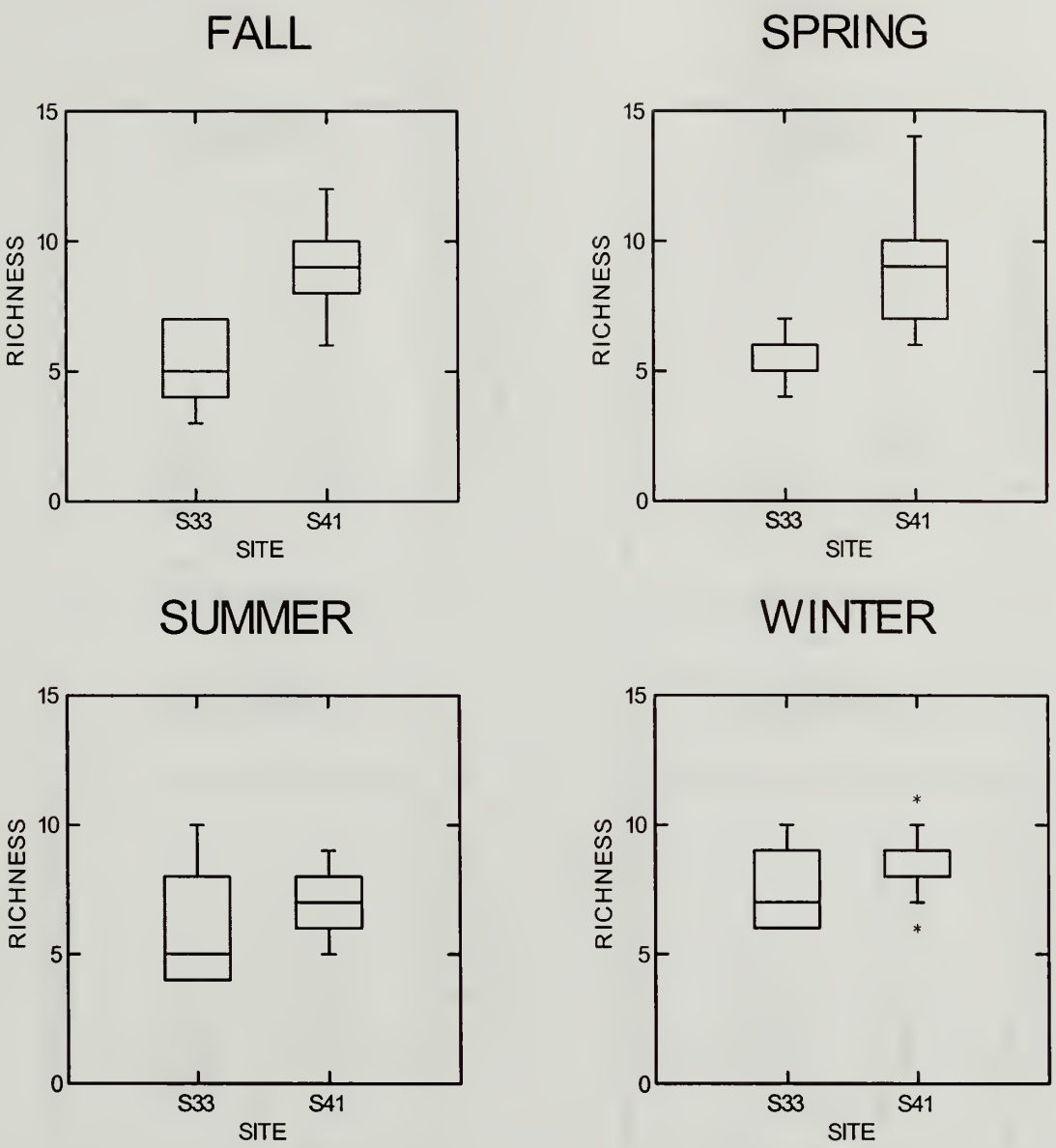

Figure 2. Macroinvertebrate taxa richness comparison between Gilbert Spring (S41) and Mitch Hill Spring (S33) for four seasons (2000/2001).

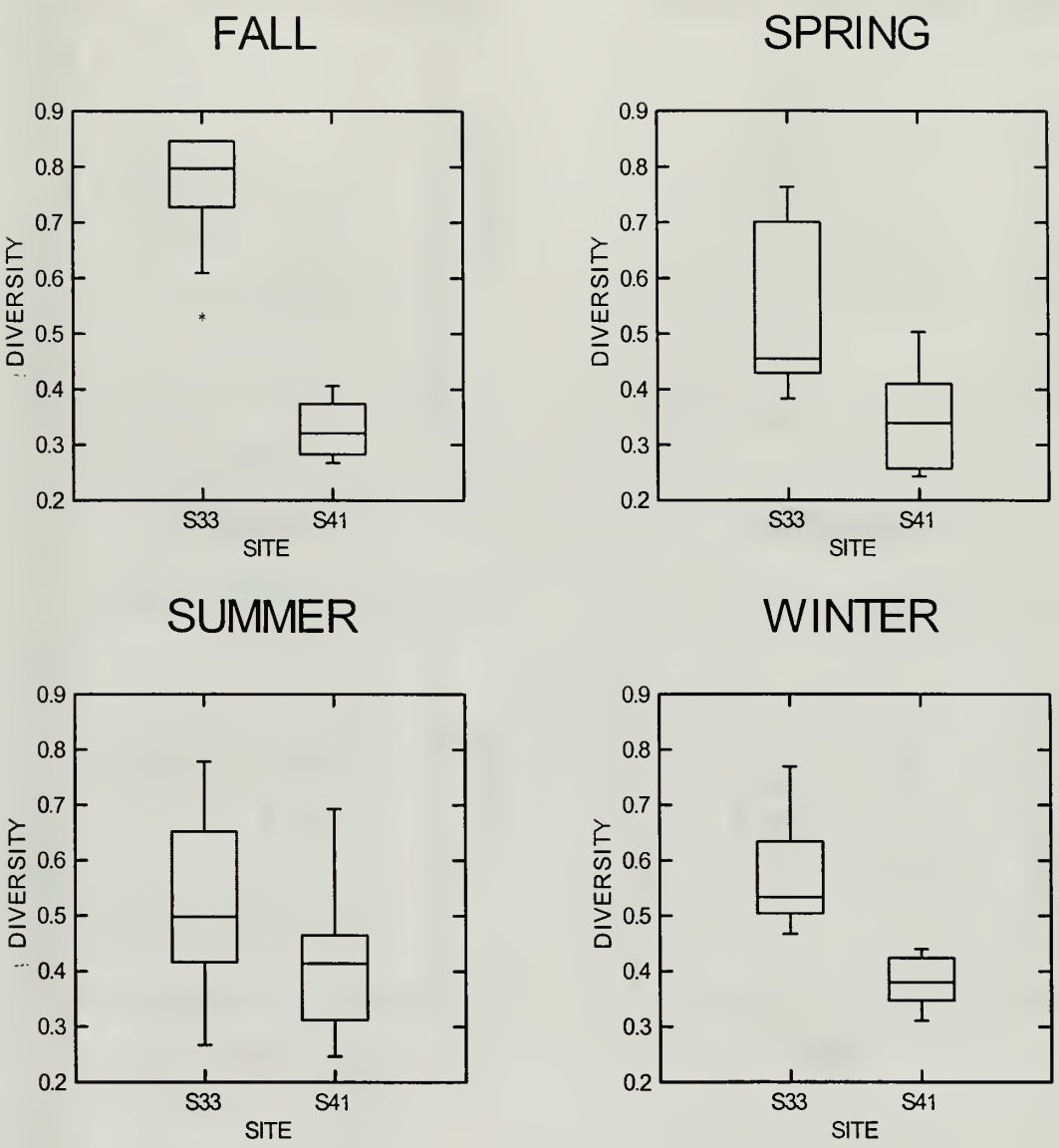

Figure 3. Macroinvertebrate community diversity (Simpson's Index) for Gilbert Spring (S41) and Mitch Hill Spring (S33) for four seasons.

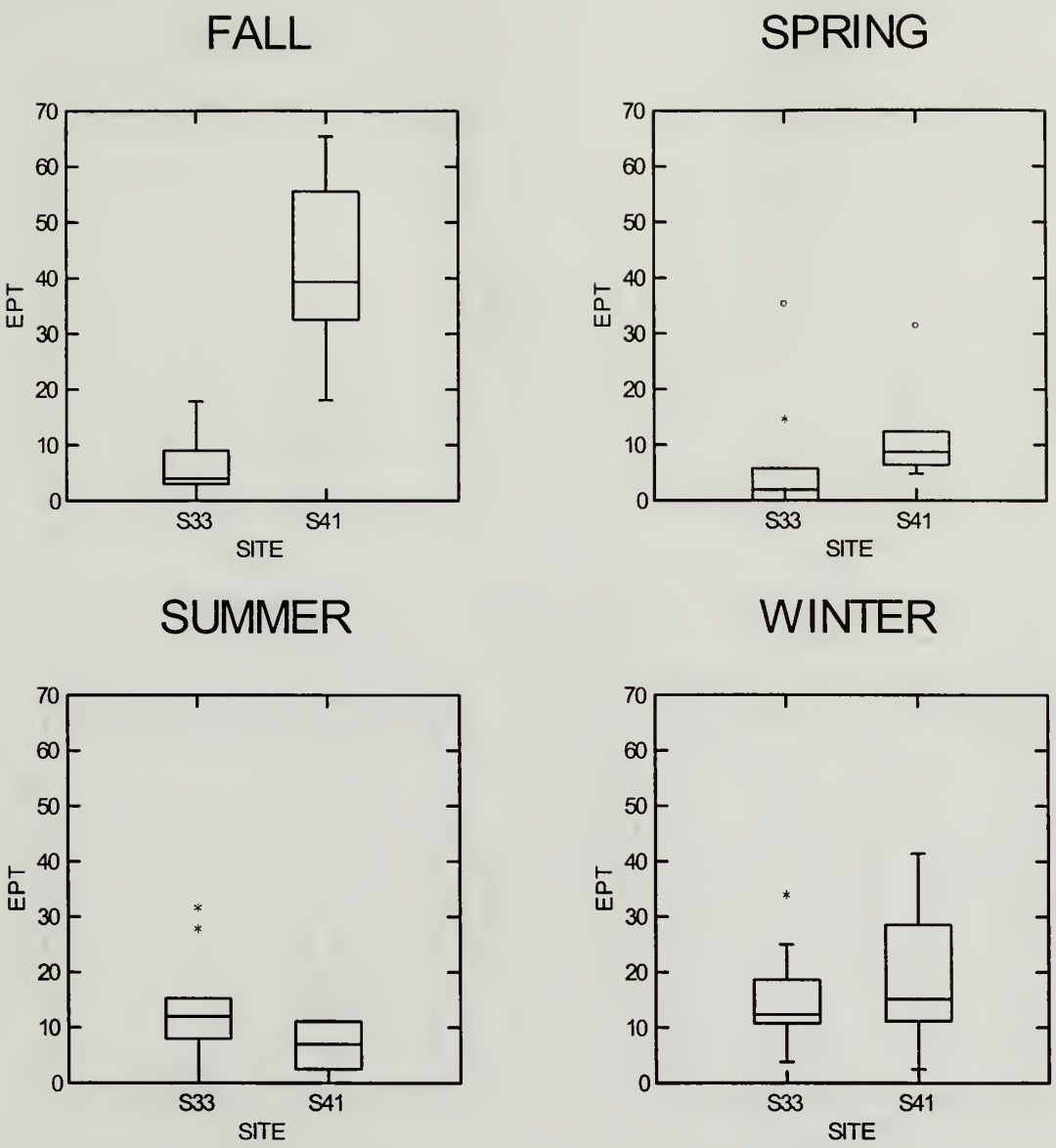

Figure 4. Percent Ephemeroptera/Plecoptera/Trichoptera (EPT) at Gilbert Spring (S41) versus Mitch Hill Spring (S33) during four seasons.

Figure 5. Percent Diptera at Gilbert Spring (S41) versus Mitch Hill Spring (S33) for four seasons.

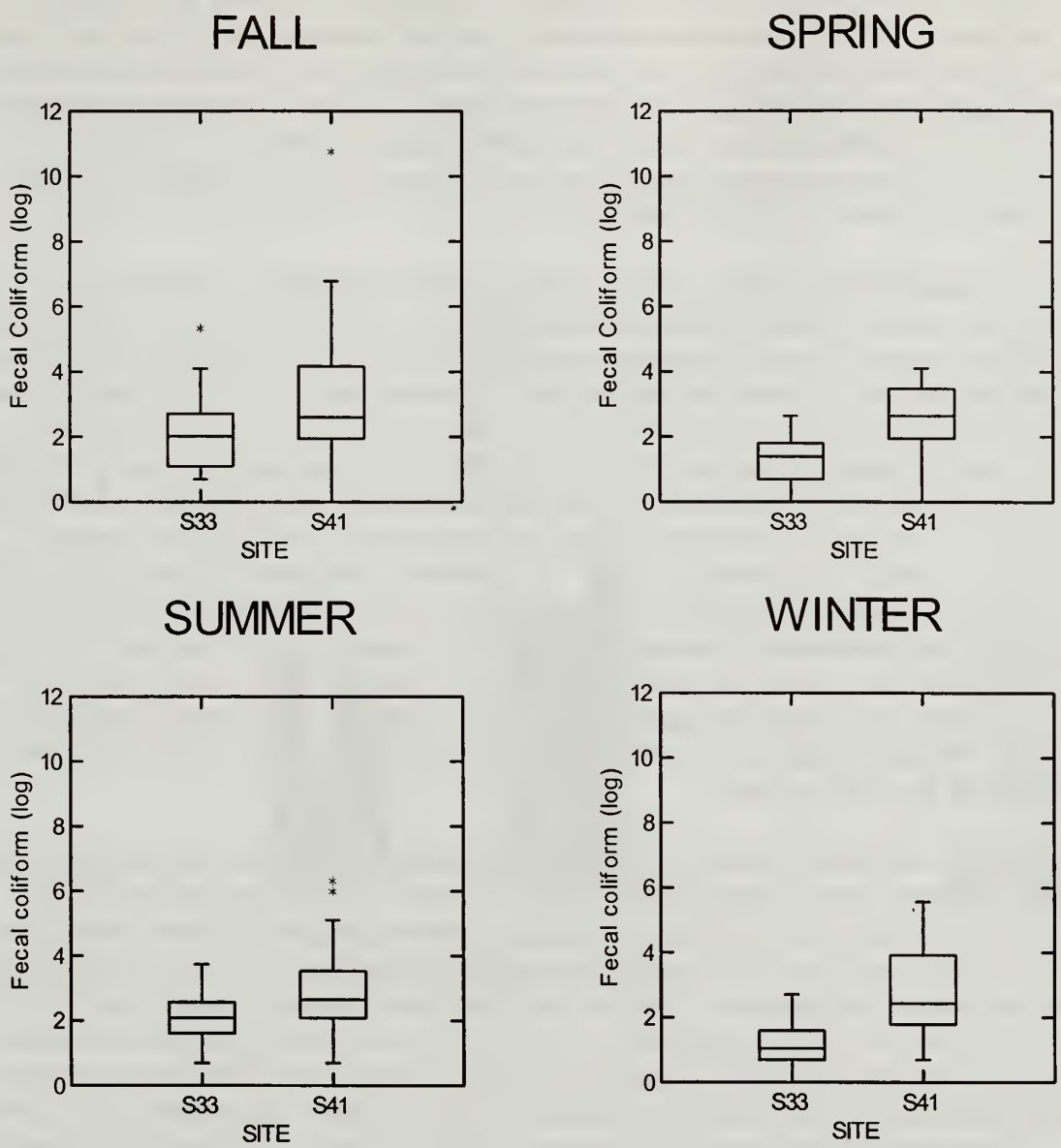

Figure 6. Fecal Coliform means by season from Gilbert Spring (S41) and Mitch Hill Spring (S33).

Physicochemical characteristics of mean temperature, conductivity (μmhos), pH, dissolved oxygen, and turbidity (NTU) were similar between the spring systems, with Gilbert Spring being slightly higher in turbidity (Table 3). Mean fecal coliform density and nitrates concentrations (NO3) were higher at Gilbert Spring for all seasons (Figure 7, Table 3). Mean orthophosphate concentrations were highest at Gilbert Spring for summer, fall, and winter. No relationships between nitrates and orthophosphates were found with macroinvertebrate community indices (seasonal mean comparison, n = 4 per system).

The physical characteristics of the two spring systems are quite dissimilar. The tail waters that create the fluvial habitat at Gilbert Springs are quite short in length, approximately 530 feet. The system at Gilbert Springs is totally contained within the Buffalo River flood plain, and subject to seasonal, backwater flooding from the Buffalo River. Alternatively, Mitch Hill Spring is located approximately $1/8^{th}$ of a mile from the Buffalo River, and well above the river's flood plain. Mitch Hill is also bordered on the west by a gravel road that extends nearly the entire length of fluvial system. Mitch Hill was deeper during all seasons sampled and had higher velocities, with the exception of spring season (Table 4). Gilbert Spring was considerably higher in mean percent canopy coverage for all seasons (Table 4). Substrate sizes were typically larger at Mitch Hill, but substrate diversity was higher at Gilbert Spring, with the exception of fall (Table 4). After the spring sampling and prior to summer sampling, a large flood event occurred within the Buffalo River watershed. Based on visual observations of effect after the flood, Mitch Hill Spring had lost most of the attached vegetation, and the vegetation was displaced downstream. Gilbert Spring was inundated with fine sediment and organics, as a result of its location within the flood plain of the Buffalo River.

Two-way analysis of variance (ANOVA) resulted in the macroinvertebrate communities being significantly different for the metrics of % Diptera, % Gammarus, % Agapetus, and ICI (Bonferroni's post hoc test for significance, Table 5). The two categorical factors used in the analysis were "sites" and "season". Interactions between the factors of "site" and "season" were significant for three of the community indices; Taxa Richness, Species Diversity, and % EPT (p-values: 0.024, 0.001, and 0.000, respectively, Table 5). The interaction of site and season for these indices indicate that the effect of season is an integral part of the effect of site; therefore, the determination of difference cannot be made for the effect of sites independent of the effect of season. All dependent variables used in the two-way ANOVA were examined for independence, constant variance, and normality.

Pearson's test for correlation found no relationships between physical habitat measurements and macroinvertebrate community indices at Mitch Hill Spring. Gilbert Spring's macroinvertebrate community had taxa richness positively related to bottom velocity ($r = 0.491$, p-value 0.024, $n = 36$) and to substrate size ($r = 0.532$, p-value 0.008, $n = 36$). Correlative coefficients were generated at the highest scale, the two systems combined ($n = 72$), and macroinvertebrate diversity was positively related to depth ($r = 0.503$, p-value 0.000) and substrate size ($r = 0.416$, p-value 0.003).

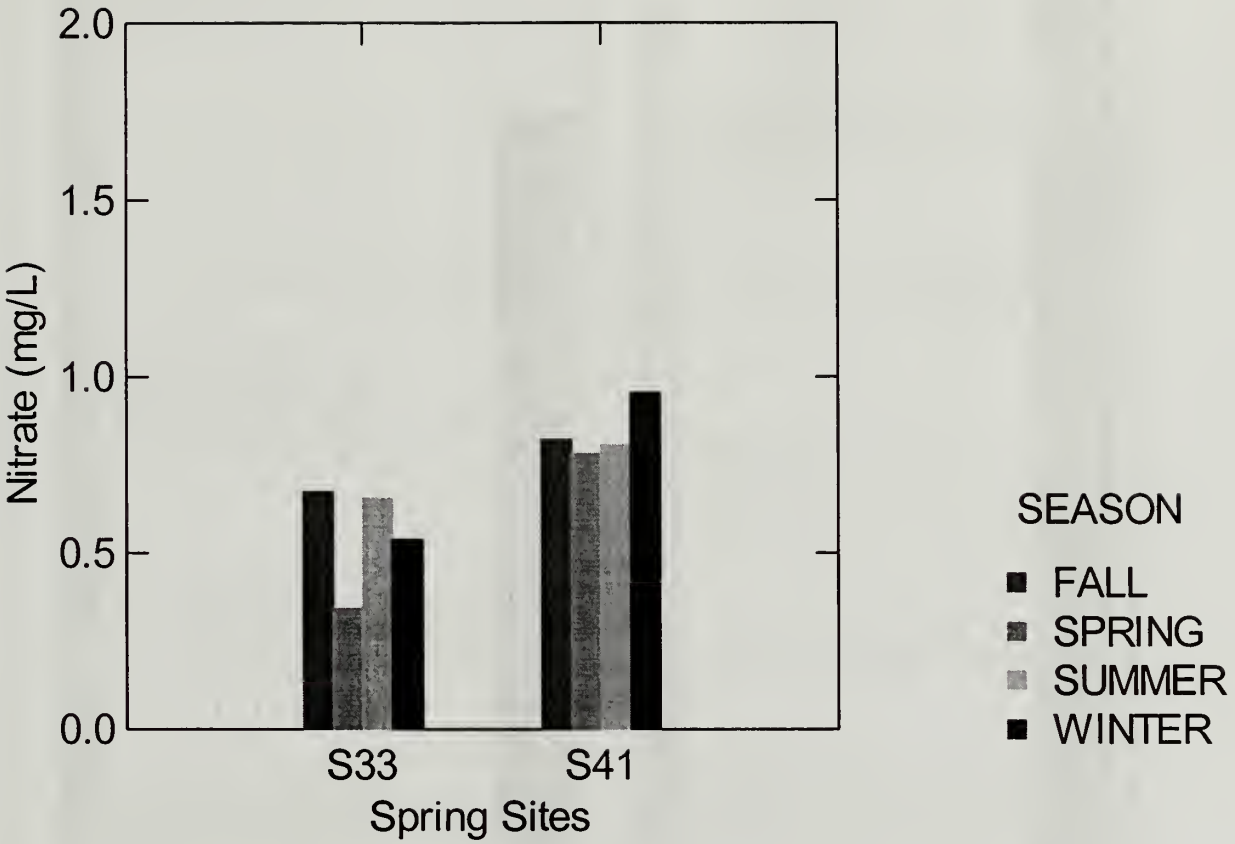

Figure 7. Seasonal nitrate (NO_3-N) concentrations (mg/L) from Gilbert Spring (S41) versus Mitch Hill Spring (S33).

Table 3. Seasonal means from physicochemical parameters collected from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Temperature	Conductivity (mhos)	pH	Dissolved Oxygen	Turbidity	Fecal Coliform	Nitrates (NO_3-N)	Orthophosphates (PO_4-P)
S33	Spring	13.4	309.5	7.4	8.4	0.9	4.2	0.341	0.040
S33	Summer	15.6	357.9	7.3	7.1	1.1	10.2	0.655	0.025
S33	Fall	15.0	385.6	7.3	7.8	1.1	22	0.673	0.030
S33	Winter	13.2	335.0	7.5	9.3	1.0	3.9	0.537	0.025
S41	Spring	13.3	346.7	7.6	8.7	1.4	21.1	0.781	0.028
S41	Summer	17.0	385.2	7.6	7.6	1.0	60.2	0.805	0.034
S41	Fall	15.4	383.1	7.2	9.0	1.6	39.1	0.821	0.040
S41	Winter	11.4	327.2	7.6	10.3	1.3	47.5	0.954	0.032

Table 4. Seasonal means for physical habitat parameters collected from Mitch Hill Spring (S33) and Gilbert Spring (S41).

Site	Season	Depth (ft)	Velocity (ft/s)	Percent Canopy	Substrate Diversity	Substrate Size (mm)
S33	Spring	0.6	0.6	46	0.381	36
S33	Summer	0.5	0.8	65	0.371	42
S33	Fall	0.5	0.5	64	0.417	38
S33	Winter	0.6	1.4	45	0.379	37
S41	Spring	0.4	1.1	95	0.444	29
S41	Summer	0.2	0.6	100	0.393	18
S41	Fall	0.2	0.4	98	0.410	24
S41	Winter	0.3	0.8	80	0.499	18

Table 5. Bonferroni probability values from the Two-way ANOVA for Gilbert and Mitch Hill's macroinvertebrate communities. Dependent variables are based upon individual metrics and the integrative Index of Community Integrity (ICI). Unevenness in sample size is due to low value log transformations.

Dependent Variable	n	Seasons (degrees of freedom)	Sites (degrees of freedom)	Seasons*Sites
Taxa Richness	72	0.087(3)	**0.000**(1)	**0.024**
Species Diversity	72	0.068(3)	**0.000**(1)	**0.001**
% EPT (Log transformed)	65	**0.039**(3)	**0.006**(1)	**0.000**
% Diptera (Log transformed)	59	0.140(3)	**0.000**(1)	0.126
% Gammarus	72	0.087(3)	**0.000**(1)	0.062
%Agapetus (Log transformed)	50	0.440(3)	**0.006**(1)	0.068
IC1	23	0.360(3)	**0.006**(1)	0.555

Table 6. Spearman's results from spring systems exhibiting potential relationships with physical habitat.

Site	Dependent	Independent	Spearman's Coefficient	Probability Value
Mitch Hill Spring	Species Diversity	Substrate Diversity	+ 1.000	< 0.05
Mitch Hill Spring	% Diptera	Substrate Diversity	- 1.000	< 0.05
Mitch Hill Spring	% Gammarus	Substrate Diversity	+ 1.000	< 0.05
Mitch Hill Spring	% Agapetus	Substrate Size	- 1.000	< 0.05
Mitch Hill Spring	Nitrates	No biotic variable		
Mitch Hill Spring	Orthophosphates	No biotic variable		
Gilbert Spring	% Gammarus	Substrate Diversity	+ 1.000	< 0.05
Gilbert Spring	Nitrates	No biotic variable		
Gilbert Spring	Orthophosphates	No biotic variable		

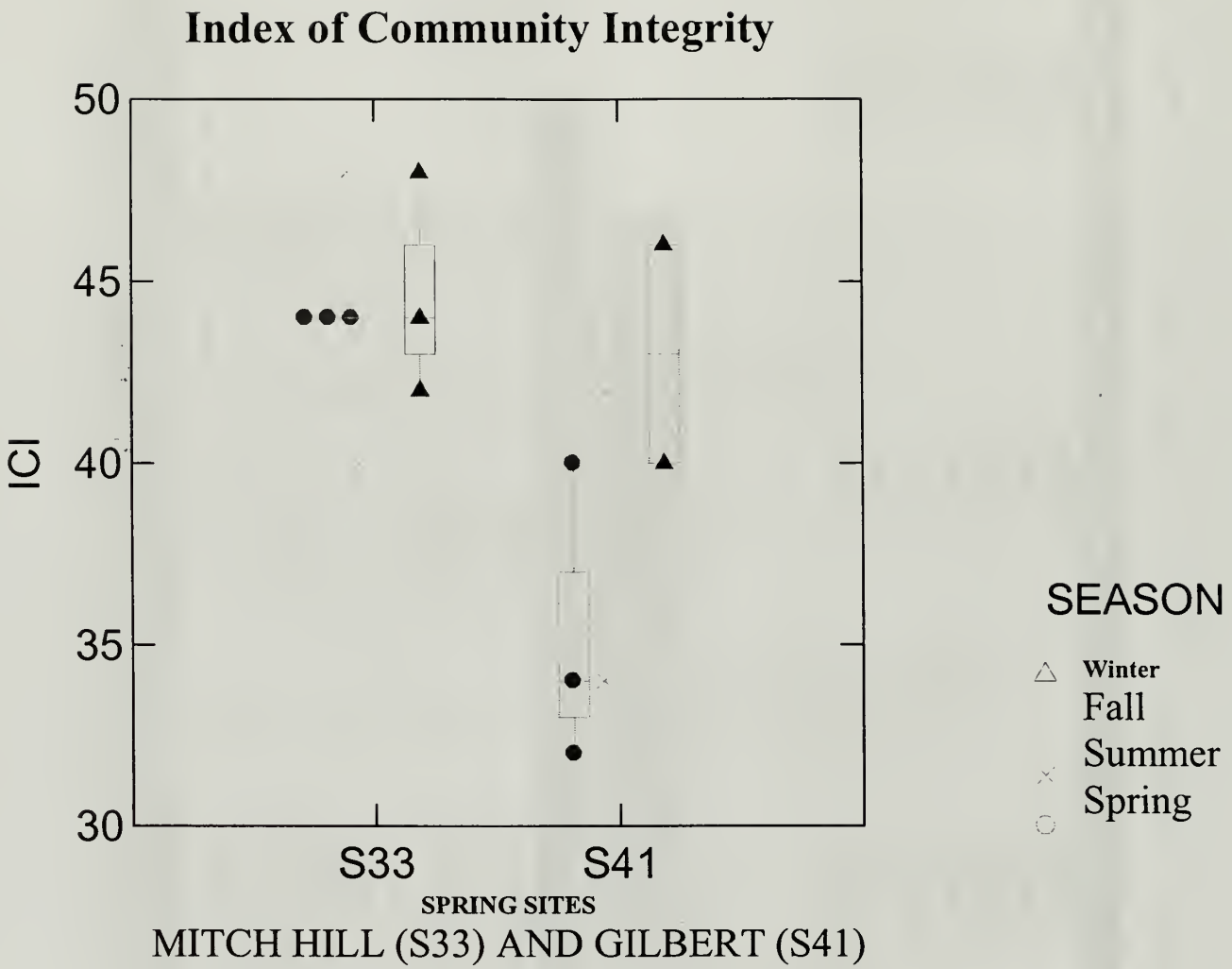

Figure 8. Comparison of Gilbert and Mitch Hill springs using the Index of Community Integrity. Box plot distributions are supplemented with symmetrical dot densities.

Discussion

Nutrient spiraling within streams describes the passage of an atomic element from an aquatic phase where it exists as a dissolved available nutrient, through its incorporation into living tissue (biotic phase) and possible passage through several links in the food chain before its eventual release into the water by excretion and/or decomposition (Allan, 1996). In most streams, biological uptake and assimilation of nutrients into organisms is accomplished primarily by autotrophs such as aquatic macrophytes, phytoplankton, and epilithic periphyton. Assimilation rates of dissolved nutrients by periphyton are influenced by the availability of nutrients and physical environmental parameters such as light, space, stream velocity, and water temperature. Aquatic community response to a moderate increase in nutrients will typically include an increase in periphytic density followed by shifts in macroinvertebrate community structure toward herbivory and the grazing functional feeding group (Allan, 1996).

Gilbert Spring was considerably higher in nutrients than Mitch Hill Spring, but no correlations between increasing nutrients and decreases in community indices or the ICI were found. The only herbivore that was consistently found between the two sites was *Agepetus*, which had higher percentages in Gilbert Spring (Table 2). Also of interest was the higher abundance of *Cheumatopsyche* in Gilbert Spring. *Cheumatopsyche* has a tolerance value of 6.6, and *Agepetus* has a value of 0, little or no tolerance of pollution (Southeast, Barbour et. al., 1999). This suggests that both pollution tolerant and intolerant genera of Trichoptera are existing within the system at Gilbert Spring and higher nutrients are not directly affecting the individuals or the community structure, although the evidence for the later is not conclusive. This result seems paradoxical when considering the higher nutrient concentrations found in Gilbert Spring.

The uptake of nutrients by autotrophs is controlled primarily by the availability of light, and other physical parameters (as discussed above) could be considered as secondary. Gilbert Spring was considerably more shaded than Mitch Hill Spring (Table 6). When considering the lack of direct sunlight and the shortness in system length, nutrient uptake may be limited in the Gilbert Spring system. This lack of ability of the aquatic community to fix energy biologically might explain the lack of community change based upon effects of poorer water-quality. Another explanation could be the collapsed sample size used within the statistical process of correlation. In order to couple the community indices with water-quality measurements the variability was reduced into a mean seasonal value. These seasonal mean values were then examined for variation relationship strengths with the individual indices and ICI. With the collapsed data set, the sample size was four per system, which requires a near perfect, succinct relationship in order to achieve statistical significance. This alone could explain the lack of correlative values between poor water-quality and declines in the macroinvertebrate community.

Taxa richness is typically expected to decrease within the macroinvertebrate community as general perturbation increases (Barbour et. al., 1999), and taxa richness was found to be higher in Gilbert Spring. This result should not be weighted too heavily when comparing the two systems based upon water-quality or the quality of the physical habitat. Generally, taxa richness was only higher at Gilbert Spring by one or two taxa, with few or only one individual representing that taxa group, depending upon season. Taxa richness within Gilbert Spring exhibited a positive

relationship with increased bottom velocity and substrate size. This would indicate that a more suitable habitat was created by these two physical gradients. Another aspect that should be considered is the close proximity of the Buffalo River to the habitats within Gilbert Springs. The taxa richness found within the Buffalo River at the mouth of Gilbert Spring could be two or three times higher than found within Gilbert Spring. The closeness of the larger species pool to the community within Gilbert Spring could account for the higher taxa richness in Gilbert Spring than Mitch Hill Spring by physical setting alone. Evidence to support this explanation can be found in the presence of the caddisflies *Leptostoma* and *Chimarra* within Gilbert Spring. The collector-gatherers are commonly found within the river corridor, and were not expected to be found within a hypocrenal system.

Macroinvertebrate community diversity, as depicted by Simpson's index, was consistently lower in Gilbert Spring throughout all the seasons. No relationships were found with poorer water-quality, physical habitat, and macroinvertebrate community indices within Gilbert Spring. However, at a larger scale (the two systems combined, n = 72), potential relationships were found with increasing depth and substrate size. Perhaps, this suggests that Gilbert Spring was lower in diversity because it had more shallow habitats with smaller substrate sizes. This conclusion is logical based upon the location of the two systems. The entire length of the Gilbert Spring system lies within the flood plain of the Buffalo River and is subject to yearly inundation. With this seasonal flooding, sediments are deposited within the active hypocrenal channel covering the larger sediments and creating a physical habitat with smaller substrate types. Alternately, Mitch Hill Spring is located within a drainage basin system and channel morphology is maintained by flood events that occur within that drainage network. Mitch Hill Spring substrate size could also be increased by it's proximity to a gravel road were larger substrates are washed into the Mitch Hill system.

The Index of Community Integrity was especially designed for the Buffalo River and it's tributaries based upon seasonal water-quality values (i.e. nitrate concentrations) and macroinvertebrate collections over a period of 3 to 4 years. The ICI uses 10 metrics that examine the various characteristics of the macroinvertebrate community structure, as recommended by Barbour et al. (1999). Established ranges for each metric was normalized by assigning scores of 2 (for data of the poorest quality), 4, 6, and 8 (for data indicating various intermediate levels of water quality), and 10 (for data indicating best water quality). By normalizing in this manner, the effects of different measurement units and ranges in values can be eliminated, and no one metric is inherently more influential than any of the others. Once accomplished, scores for all metrics are summed and a total ICI score is generated.

Gilbert Spring was lower in ICI scores for all seasons. The ICI scoring system was created upon ranges in water-quality known to exist within the Buffalo River and it's tributaries, but the predictive properties for the individual metrics were originally designed to show changes within the macroinvertebrate community by general perturbation (Barbour, et al., 1999), which allows this biomonitoring program a dual utility. Statistical evidence indicates that the two communities are different based upon ICI scores, with Mitch Hill receiving the higher scores. Possible explanations for the difference between the communities could not be individually validated; however, the combination of poorer water-quality, differences in the quality of the

habitat, and the physical setting (i.e. general perturbation) were most probably the factors that lead to Gilbert Spring having lower ICI scores.

Conclusions

Predictions of declining macroinvertebrate communities as a result of poorer water-quality within Gilbert and Mitch Hill Springs could not be validated. Gilbert Spring had higher nutrient values than did Mitch Hill Spring, but the disconnection of nutrients from the aquatic community due to physical shading within the habitat and low sample size most probably prevented correlative evidence from being elucidated. Based upon the ICI scoring system, Gilbert Springs had lower scores, which indicates that Gilbert Spring has a higher level of perturbation, but no individual factor was suggested by correlative efforts. Taxa richness was higher within the system of poorer water-quality. The increased taxa richness was attributed to the closeness of a larger species pool, and was not considered a product of the spring system. Macroinvertebrate community diversity was found to be much lower at Gilbert Spring, and a relationship was found that suggests diversity is negatively effected by smaller substrate sizes found within Gilbert Spring. This conclusion was supported by field observations and the positioning of the two spring systems as related to the Buffalo River's flood plain and the gravel road, which was responsible for the differences in substrate size and diversity between the two spring systems.

Section III
Water Quality Analysis

Gilbert Spring Study
Water Quality Evaluation
by
Martin Maner, P.E.
Arkansas Department of Environmental Quality

INTRODUCTION

Water quality parameters were compiled and interpreted to determine if Dry Creek and/or the septic tanks in the town of Gilbert were having an impact on Gilbert Spring. Earlier sampling by the National Park Service revealed that the spring had high bacteria counts following storm events and other water quality issues as discussed earlier.

For this study, samples were collected by the Buffalo National River personnel from February of 2000 to May of 2001. A storm event on November 6 and 7, 2000 was sampled over the course of the storm to determine storm related effects. A total of 43 parameters with over 2,300 individual readings were taken and evaluated for this study.

Samples were collected from Dry Creek at the Highway 333 bridge (BUFT11.5), Gilbert Spring at the outflow (BUFS41), Buffalo River above Dry Creek (BUFR5.9), and Buffalo River below the confluence of Gilbert Spring (BUFR6.1). This last station is also below Dry Creek, which as the name implies, is dry under base-flow conditions from 200 yards below the Highway 333 bridge to the confluence with the Buffalo River (see Appendix B1).

Parameters included the following: flow, dissolved oxygen, water temperature, pH, alkalinity, conductivity, ammonia-nitrogen, nitrite/nitrate-nitrogen, total kjeldahl-nitrogen, total phosphorus, ortho phosphorus, total organic carbon, biochemical oxygen demand, turbidity, total suspended solids, total dissolved solids, hardness, silica, bromides, chlorides, fluorides, sulfates, aluminum, arsenic, barium, beryllium, boron, cadmium, calcium, total chromium, cobalt, copper, iron, lead, magnesium, manganese, nickel, potassium, selenium, sodium, vanadium, and zinc.

SUMMARY

Based on the water quality evaluation, primarily chlorides and manganese data, Gilbert Spring is significantly influenced by surface in-flow. Water quality is very similar between Dry Creek and Gilbert Spring. The data suggests that the major impact on Gilbert Spring is by Dry Creek. Based on interpretation of the chlorides data, it appears that the septic tanks are having little impact on Gilbert Spring.

Fecal coliform bacteria counts exceeded the water quality standards criteria in both Dry Creek and Gilbert Spring during a storm event in November of 2000. Fecal coliform values in Dry Creek reached 174,600 colonies/100 ml and 46,800 colonies/100 ml in Gilbert Spring. If storm event monitoring had continued, we believe bacteria counts in Gilbert Spring would have eventually approached the values seen in Dry Creek, since dye tracing has confirmed a direct and open connection between the creek and spring.

The total phosphorus guideline of 100 ug/l was exceeded in Dry Creek and Gilbert Spring during this same storm event, with maximum concentrations of 138 ug/l in Dry Creek and 108 ug/l in Gilbert Spring.

A dissolved oxygen value of 5.3 mg/l was recorded in the upstream sample in the Buffalo River (BUFR5.9) on July 17, 2000. This site is at the end of a long pool. At the downstream sample site (BUFR6.1), which is in a riffle area, the dissolved oxygen was 6 mg/l. The numeric criteria is 6 mg/l. The low dissolved oxygen at the upstream sample is most probably caused by the benthal oxygen demand. The dissolved oxygen was also low in Dry Creek on several occasions. This was also probably due to a high benthal oxygen demand.

These water quality impacts of elevated bacteria, phosphorus, and low dissolved oxygen are most likely due to non-point source runoff. The Dry Creek basin is included within a Natural Resources Conservation Service (NRCS) sponsored watershed protection/water quality improvement program which is intended to reduce nonpoint source runoff impacts through the installation of voluntary Best Management Practices using federal cost-share. Water quality in Dry Creek, Gilbert Spring during storm events, and the Buffalo River could be improved with better control over the non-point sources of pollution in the respective watersheds. The most likely source of the non-point source pollutants is animal waste and un-surfaced road runoff as documented by the NRCS (USDA, 1995).

GENERAL DISCUSSION

The evaluation of these parameters included: (1) comparison of storm event and routine sampling of Dry Creek (Station BUFT11.5) and Gilbert Spring (Station BUFS41) to determine if Dry Creek or the septic tanks of Gilbert were impacting the spring, and (2) comparison of the results of routine sampling from all four stations to appropriate water quality criteria.

Applicable State Water Quality Criteria

Dry Creek and this portion of the Buffalo River are in the Ozark Highlands Ecoregion, therefore the Ozark Highland criteria apply. Chapter Five of Arkansas' water quality standards, entitled "Regulation 2, Regulation Establishing Water Quality Standards for Surface Waters of the State of Arkansas" should be referred to for specific numeric criteria. The selected criteria are presented here for comparison to survey results.

Selected Numeric Criteria

Maximum Allowable Temperature = 29 degrees Celsius

Turbidity for Streams = 10 NTU

Dissolved Oxygen for Streams

Watershed 10 to 100 square miles: Primary Season = 6 mg/l

Critical Season = 5 mg/l

Watershed > 100 square miles: Primary Season = 6 mg/l
Critical Season = 6 mg/l

Bacteria: See Section 2.507 of Reg. 2.

Dissolved Metals, Chronic Criteria (based on a hardness of 100 mg/l)

Cadmium = 1.13 ug/l
Copper = 11.82 ug/l
Lead = 3.18 ug/l
Nickel = 157.67 ug/l
Selenium = 5 ug/l
Zinc = 105.99 ug/l

Nutrients: Total Phosphorus, as a guideline, should not exceed 100 ug/l.

Mineral Quality	Chlorides	Sulfates	Total Dissolved Solids
Buffalo River	20 mg/l	20 mg/l	180 mg/l
Ozark Highland Tributary Streams	17 mg/l	23 mg/l	280 mg/l

(these values are based on ecoregion reference stream values, plus 1/3 of the value. See Section 2.511 of Reg. 2.)

RESULTS

Storm Event of November 6-7, 2000

Five sample sets were collected from Dry Creek and Gilbert Spring from approximately 2 am to about 5 pm on November 6 and one sample set at about 8 am on November 7. Flow increased from about 2 cfs in both Dry Creek and Gilbert Spring to about 7 cfs in Dry Creek and 4.5 cfs in the spring. The maximum flow measurement in the creek was at 11:05 am on the 6th and in the spring at 4:20 pm on the 6th.

Water Quality Items of Concern

Fecal Coliform bacteria showed a dramatic increase based on the samples collected on the morning of November 7. Values were 174,600 colonies/100 ml in Dry Creek and 46,800 colonies/100 ml in Gilbert Spring. The Arkansas standard for fecal coliform at this time of year for this size of watershed (see Section 2.507 (B) and (C)) is not to exceed 2000 colonies/100 ml in more than 10 percent of the samples taken in any 30-day period. Since this is one of seven samples taken within a 30 day period (Oct 16 to Nov 7), it would constitute 14.2 percent of the 7 samples taken and is therefore a water quality violation. Based on other parameters discussed below, the apparent impact to Gilbert Spring is caused by surface runoff, particularly Dry Creek.

Therefore, improving water quality in the Dry Creek watershed should also improve water quality in Gilbert Spring.

Total phosphorus exceeded the 100 ug/l guideline for nutrients in the Arkansas water quality standards (see Section 2.509) in the November 7 sample. Values were 138 ug/l in Dry Creek and 108 in Gilbert Spring.

General Items

Chlorides are a conservative substance tending not to degrade in the environment. Typical natural surface waters have chloride values of about 5 mg/l in the Ozark Highlands. Typical domestic sewage, such as from septic tanks, is about 30 mg/l. Animal manure, used as a fertilizer, also has elevated values of chlorides. If Gilbert Spring was being influenced by septic leachate, one would expect the chloride values to be elevated when compared to Dry Creek. However, during the storm event, Dry Creek chlorides were higher than Gilbert Spring, therefore one could conclude that the septic tanks are having little if any impact on the spring. Apparently, the increase in chlorides in Gilbert spring is associated with the increase in Dry Creek, that is, Dry Creek is losing most of its flow to Gilbert Spring and affecting the springs water quality. The elevated chlorides in Dry Creek observed during the storm event sampling (maximum concentration of 11.95 mg/L) could be from land application of animal manure or waste.

One interesting result of the storm event sampling was the behavior of manganese values. Manganese is a naturally occurring element associated with rock strata in the Gilbert area. At the start of the storm event sampling, manganese in Dry Creek was 6.5 ug/l and in Gilbert Spring was 16.4 ug/l. However, as the storm event sampling progressed, and flow in Gilbert Spring increased, the manganese of both the creek and spring decreased to less than 3 ug/l. This indicates that the spring is strongly influenced by surface runoff, since the initial manganese concentrations in the spring were significantly diluted. Apparently Dry Creek is a significant component of this surface influence.

Storm event results are tabulated in Table 4, and displayed graphically in Appendix E1.

Monthly/Semi-monthly Routine Sampling

Monthly or semi-monthly samples were taken of Dry Creek, Gilbert Spring, Buffalo River upstream of Dry Creek and downstream of Gilbert Spring run from February 28, 2000 to May 21, 2001.

Water Quality Standards Items of Concern

Buffalo River

Maximum water temperatures were very slightly above the ecoregion maximum of 29 degrees Celsius on July 17, 2000 in the upstream and downstream samples for the Buffalo River.

The lowest dissolved oxygen value recorded for the samples taken in the Buffalo River also occurred on July 17. The value was 5.3 mg/l (68% saturation) at the upstream site and 6.0 mg/l (77% saturation) downstream. The dissolved oxygen numeric criteria for the Buffalo River is 6.0 mg/l. The upstream site is at the end of a pool, whereas the downstream site is in a riffle. The low dissolved oxygen was probably due to the low reaeration of the pool combined with an oxygen demand from the benthal community and sediment. Apparently, the low dissolved oxygen at the upstream site was reaerated by the riffle.

These are the only water quality standard areas of concern, except for the fact that the Buffalo River is an extraordinary resource water. As such, Section 2.203, *Outstanding Resource Waters*, of the water quality standards apply. That section states in part " Where high quality waters constitute an outstanding state or national resource, such as those waters designated as extraordinary resource waters, ..., those uses and water quality for which the outstanding waterbody was designated shall be protected by (1) water quality controls, (2) maintenance of natural flow regime, (3) protection of instream habitat, and (4) pursuit of land management protective of the watershed."

Dry Creek

Dissolved oxygen in Dry Creek was 4.9 mg/l (52 % saturation) on September 12, 2000. There were also two days, August 21, 2000 and October 16, 2000, when the value was 5 mg/l. Saturation for these days was 53 % and 49 % respectively. These depressed values were probably due to a high benthal and/or sediment oxygen demand, since the total organic carbon and biochemical oxygen demand values were low. High benthal and/or sediment oxygen demand is typically due to non-point source runoff in the form of animal manure. This material settles on the bottom, and has a high oxygen demand. It can also enrich the benthic flora and fauna, which will exert a higher oxygen demand.

General Items

The routine sampling showed that in general, the water quality in Dry Creek and Gilbert Spring are very similar. The only exception was the elevated turbidity and total suspended solids in Gilbert Spring compared to Dry Creek observed on November 6, 2000. This was during the storm event discussed above. Since the storm event sampling showed that the turbidity and total suspended solids in Gilbert Spring became elevated before turbidity was elevated in Dry Creek, it would indicate that suspended solids were being picked up in the subsurface channels of the spring, or that other surface waters besides Dry Creek were influencing the spring. Previous dye-tracing in the Gilbert area by Aley (1982) showed that a solution valley just north of Gilbert is within the recharge zone of Gilbert Spring. Road runoff was observed going into this drainage during the rain event field work. This runoff is believed to be the primary contributor of suspended sediment observed in Gilbert Spring during the early portion of the rain event study.

The routine sampling showed that the upstream and downstream water quality in the Buffalo River are, in general, similar. Metal values in the river are well below the

numeric criteria. In general, water quality in the Buffalo River is good. However, there should be some concern regarding the elevated levels of phosphorus occasionally seen and the reduced levels of dissolved oxygen. These observations are probably due to non-point source runoff.

Routine sampling results are tabulated in Table 5 and depicted graphically in Appendix E2.

Section IV
Conclusions and Recommendations

Conclusions

This study looked at the entire hydrologic framework of the Gilbert area and assessed the potential impacts of Gilbert's septic system in a comprehensive manner. This broad-spectrum assessment was made possible through the funding provided by the National Park Service, the laboratory analysis and other assistance provided by the Arkansas Department of Environmental Quality, and, most importantly, by the willingness of Gilbert's residents to allow us to come into their homes and trace from their septic systems. Water quality monitoring at Gilbert Spring over the last 15 years alerted us to a potential problem, and an assessment of the field setting indicated the septic systems in Gilbert as the most probable source. In summary, the broader studies reported here showed this assumption was mostly incorrect.

Previous dye tracing reported by Aley (1982) showed a hydrologic connection between Dry Creek and Gilbert Spring (see map in Appendix B1). In an effort to better assess this connection, a time of travel study was conducted between Dry Creek and Gilbert Spring that showed a very open ground water conduit existed between the losing portion of Dry Creek and Gilbert Spring. Monthly or by-monthly flow measurements taken from Dry Creek and Gilbert Spring showed that often, Gilbert Spring's flow can be completely accounted for by the flow being lost from Dry Creek. During rain events, surface runoff is pirated by nearby solution valleys and also contributes flow to Gilbert Spring. However, during base-flow conditions, Gilbert Spring is little more than the resurgence of Dry Creek.

We completed over 30 individual dye traces from 29 septic systems in the town of Gilbert (85 percent of the targeted systems). We had two major objectives defined for these traces: 1.) determine if septic systems constructed in accordance with Arkansas State Regulations for septic systems are capable of functioning in the karst setting of Gilbert, and 2.) find any systems which are contributing leachate directly to Gilbert Spring. To address objective 1, we traced seven of the best septic systems identified in Gilbert simultaneously using two types of dye. Very small amounts of fluorescein were recovered from these traces, and we conclude that these systems are over 98 percent efficient in removing the tracer dye from the leachate. Our conclusion is that septic systems can and do work well in Gilbert's karst setting where they are properly constructed.

To address objective two we traced the remaining 22 septic systems using four types of tracer dyes over a three month period. Two systems were found to rapidly contribute dye directly to Gilbert Spring in large quantities. Two other systems contributed measurable but minor quantities of dye to Gilbert Spring, and the remaining 18 systems contributed negligible amounts of dye to Gilbert Spring. Of the two systems that contributed leachate directly to Gilbert Spring, one was found to be discharging to an old cistern, and the other was found to have a broken pipe running from the house to the septic tank. The system using the old cistern was completely replaced through cost-share assistance provided by Buffalo National River, and the system with the broken pipe was repaired by the landowner.

The biologic community within Gilbert Spring's run (the stream formed below the spring) was assessed by comparing the macroinvertebrate community from the run with the macroinvertebrate community in a nearby, similar spring run formed below Mitch Hill Spring. The macroinvertebrate community was also assessed by performing various community metrics and comparing the results with results from other studies and information. These studies indicate that the Gilbert Spring run is a unique system with regard to its physical location in the Buffalo River floodplain and the frequent inundation with backwater when the river floods. In general, the aquatic community within Gilbert Spring's run was not significantly impacted by leachate from Gilbert's septic systems.

Water quality analyses performed as part of this study confirmed problems with fecal coliform bacteria, phosphorus, and dissolved oxygen values in Gilbert Spring at certain times. However, the source of these problems was clearly tied to Dry Creek as opposed to the septic systems in Gilbert. A report by the Natural Resources Conservation Service (USDA, 1995) found that 53 percent of the Dry Creek basin is in pasture, and that 79 percent of this pasture is considered to be "problem areas." The report also estimated 557 head of cattle in the Dry Creek basin and identified four dairy operations. Figure 4 shows the computer estimated sources of fecal coliform bacteria in the Buffalo River from the various sources identified by the Natural Resources Conservation Service during their investigation of water quality issues in the middle portion of the Buffalo River watershed, including Dry Creek. Based on the NRCS model, they estimated "1/2 of one percent of the fecal coliform bacteria transported to the River emanates from faulty septic systems." Our conclusions based on the results of this investigation are in general agreement with that estimate.

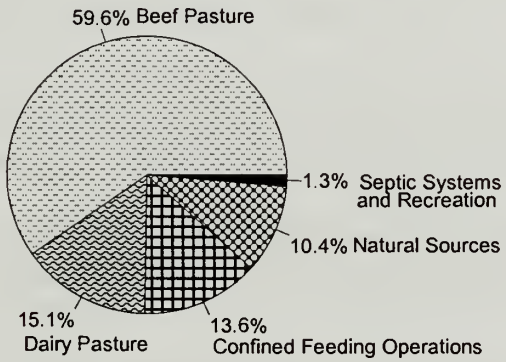

Figure 4. Sources of fecal coliform bacteria in the Buffalo River from sources in the Middle Tributaries Project Area (NRCS, 1995).

The majority of the water quality problems identified in Dry Creek and Gilbert Spring were relegated to nonpoint source pollution from agricultural operations in the Dry Creek basin. Whereas septic systems are relatively easy and inexpensive to replace or repair, nonpoint source pollution problems from agricultural operations are extremely difficult to address. Dry Creek is currently listed as a targeted basin for implementation of Best Management Practices (BMPs) under a voluntary cost-share program administered by the Natural Resources Conservation Service (USDA, 1995). Landowners in the Dry Creek basin are eligible for 75% cost-share assistance to implement such BMPs as filter strips, waste storage structures, pasture and hay management, waste management systems, conservation easements, livestock exclusion and watering facilities, fencing, streambank protection, and critical area planting.

Recommendations

1.) Continue to use septic systems for disposal of human waste in the town of Gilbert. It is critical that new systems be installed in conformance with Arkansas Regulations. Existing systems should be properly maintained, including routine pumping of tanks.

2.) Continue voluntary cost-share incentives and implementation of Best Management Practices in the Dry Creek basin to alleviate water quality problems documented in Dry Creek and Gilbert Spring.

3.) Continue routine water quality monitoring of Gilbert Spring to assess any changes in water quality in the Dry Creek basin.

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Appendix A
Ozark Underground Laboratory Quality Control/Quality Assurance

PROCEDURES AND CRITERIA
ANALYSIS OF FLUORESCEIN, EOSINE, RHODAMINE WT, SULFORHODAMINE B, AND PYRANINE DYES IN WATER AND CHARCOAL SAMPLERS

January 2, 2001

Thomas Aley, PHG 179
President
Ozark Underground Laboratory, Inc.

PROCEDURES

Introduction

This document describes standard procedures and criteria currently in use at the Ozark Underground Laboratory as of the date shown on the title page. Some samples may be subjected to different procedures and criteria because of unique conditions; such non-standard procedures and criteria are identified in reports for those samples. Standard procedures and criteria change as knowledge and experience increases and as equipment is improved or up-graded. A summary of changes in standard procedures and criteria is maintained by the Ozark Underground Laboratory.

Dye Nomenclature

Fluorescein is C.I. Acid yellow 73, Color Index Number 45350. Rhodamine WT is Acid Red 388; there is no assigned Color Index Number for this dye. Eosine (sometimes called eosin) is Acid Red 87, Color Index Number 45380. Sulforhodamine B is C.I. Acid Red 52, Color Index Number 45100. Pyranine is Solvent Green 7 (also called D&C Green 8), Color Index Number 59040.

Description of the Samplers

The charcoal samplers are packets of fiberglass screening partially filled with approximately 4.25 grams of activated coconut charcoal. The charcoal used by the Ozark Underground Laboratory is Barnebey and Sutcliffe coconut shell carbon, 6 to 12 mesh, catalog type AC.

The most commonly used samplers are about 4 inches long by two inches wide. A cigar-shaped sampler is made for use in very small diameter wells (such as 1 inch diameter wells); this is a special order item and should be specifically requested when it is needed. All of the samplers are closed by heat sealing.

Placement of Samplers

Samplers (also called charcoal packets) are placed so as to be exposed to as much water as possible. In springs and streams they are typically attached to a rock or other anchor in a riffle area. Attachment of the packets often uses plastic tie wires. In swifter water galvanized wire (such as electric fence wire) is often used. Other types of anchoring wire can be used. Electrical wire with plastic insulation is also good. Packets are attached so that they extend outward from the anchor rather than being flat against it. Two or more separately anchored packets are typically used for sampling springs and streams. The use of fewer packets is discouraged except when the spring or stream is so small that there is not appropriate space for placing multiple packets.

When pumping wells are being sampled, the samplers are placed in sample holders made of PVC pipe fittings. Brass hose fittings are installed at the end of the sample holders so that the sample holders can be installed on outside hose bibs and water which has run through the samplers can be directed to waste through a connected garden hose.

The samplers can be unscrewed in the middle so that charcoal packets can be changed. The middle portions of the samplers consists of 1.5 inch diameter pipe and pipe fittings.

Charcoal packets can also be lowered into monitoring wells for sampling purposes. In general, if the well is screened, samplers should be placed approximately in the middle of the screened interval. Some sort of weight should be added near the charcoal packet to insure that it will not float. The weight should be of such a nature that it will not affect water quality. One common approach is to anchor the packets with a plastic cable tie to the top of a dedicated weighted disposable bailer. We typically run nylon cord from the top of the well to the charcoal packet and its weight. Nylon fishing line should not be used since it can be readily cut by a sharp projection in the well.

In some cases, especially with narrow wells and appreciable well depths, the weighted disposable bailers sink very slowly or may even fail to sink because of friction and floating of the anchoring cord. In such cases a stainless steel weight may be added to the top of the disposable bailer. We have had good success with two to three ounce segments of stainless steel pipe which have an outside diameter of 1.315 inches and an inside diameter of 1.049 inches; such pipe weighs about 1.7 pounds per linear foot. The weight of the stainless steel is approximately 497 pounds per cubic foot. The pipe segments can be attached over the anchoring cord at the top of the bailer. All weights should be cleaned prior to use; the cleaning approach should comply with decontamination procedures in use at the project site.

Placement of samplers requires adjustment to field conditions. The above placement comments are intended as guidance, not firm requirements.

Rinsing of Charcoal Packets Prior to Sampling

Charcoal packets routinely contain some fine powder which washes off rapidly when they are placed in water. Since such material could remain in monitoring wells, charcoal packets to be placed in such wells are triple rinsed with distilled, demineralized, or reagent water known to be free of tracer dyes. This rinsing is typically done by soaking. With this approach, approximately 25 packets are placed in one gallon of water and soaked for at least 10 minutes. The packets are then removed from the water and excess water is shaken off the packets. The packets are then placed in a second gallon of water and again soaked for at least 10 minutes. After this soaking they are removed from the water and excess water is shaken off the packets. The packets are then placed in a third gallon of water and the procedure is again repeated. Rinsed packets are placed in plastic bags and are placed at sampling stations within three days. Packets can also be rinsed in jets of water for about one minute; this requires more water and is typically difficult to do in the field with water known to be free of tracer dyes.

Collection and Replacement of Samplers

Samplers are routinely collected and replaced from each of the sampling stations. The frequency of sampler collection and replacement is determined by the nature of the study. Collections at one week intervals are common, but shorter or longer collection frequencies are acceptable and sometimes more appropriate. Shorter sampling frequencies are often used in the early phases of a study to better characterize time of

travel. As an illustration, we often collect and change charcoal packets 1, 2, 4, and 7 days after dye injection. Subsequent sampling is then weekly.

Where convenient, the collected samplers should be briefly rinsed in the water being sampled. This is typically not necessary with well samples. The packets are shaken to remove excess water. Next, the packet (or packets) are placed in a plastic bag (Whirl-Pak bags are ideal). The bag is labeled on the outside with a permanent type felt marker pen. Use only pens that have black ink; colored inks may contain fluorescent dyes. The notations include station name or number and the date and time of collection. Labels are not inserted inside the sample bags.

For most projects the Ozark Underground Laboratory supplies the Whirl-Pak bags. Prior to use, 1% of the new bags are randomly selected. Each bag is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

Collected samplers are kept in the dark to minimize algal growth on the charcoal prior to analysis work. We prefer (and in some studies require) that samples be placed on "blue ice" or ice upon collection and that they be shipped refrigerated with "blue ice" by overnight express. Do not ship samplers packed in ice since this can create a potential for cross contamination when the ice melts. Our experience indicates that it is not essential for samplers to be maintained under refrigeration, yet maintaining them under refrigeration clearly minimizes some potential problems. A product known as "green ice" should not be used for maintaining the samples in a refrigerated condition since this product contains a dye which could contaminate samples if the "green ice" container were to break or leak.

New charcoal samplers are routinely placed when used charcoal packets are collected. The last set of samplers placed at a stream or spring is commonly not collected.

Water samples are often collected. They should be collected in either glass or plastic; the Ozark Underground Laboratory routinely uses 50 ml research grade polypropylene copolymer Perfector Scientific vials (Catalog Number 2650) for such water samples. The vials should be placed in the dark and refrigerated immediately after collection. They should be refrigerated until shipment. For most projects the Ozark Underground Laboratory supplies the vials. Prior to use, 1% of the new vials are randomly selected. Each vial is soaked in the standard eluting solution and then analyzed for the presence of any of the tracer dyes being used.

When water or charcoal samplers are collected for shipment to the Ozark Underground Laboratory they should be shipped promptly. We receive good overnight and second day air service from both UPS and Fed Ex; Airborne Service is excessively slow, and the Postal Service does not provide next day service to us.

Each shipment of charcoal samplers or water samples must be accompanied by a sample tracking sheet. These sheets (which bear the title "Samples for Fluorescence Analysis") are provided by the Ozark Underground Laboratory and summarize placement

and collection data. These sheets can be augmented by a client's chain of custody forms or any other relevant documentation. Figure 1 is one of our blank sample forms.

Receipt of Samplers

Samplers shipped to the Ozark Underground Laboratory are refrigerated upon receipt. Prior to cleaning and analysis, samplers are assigned a laboratory identification number. All samples are logged in upon receipt and are recorded in a bound journal.

It sometimes occurs that there are discrepancies between the chain-of-custody sheets and the actual samples received. When this occurs, a "Discrepancy Sheet" form is completed and sent to the shipper of the sample for resolution. A copy of this form is enclosed as Figure 2. The purpose of the form is to help resolve discrepancies, even when they may be minor.

Cleaning of Samplers

Samplers are cleaned by spraying them with jets of clean water. At the Laboratory we use unchlorinated water for the cleansing to minimize dye deterioration. Effective cleansing cannot generally be accomplished simply by washing in a conventional laboratory sink even if the sink is equipped with a spray unit.

The duration of packet washing depends upon the condition of the sampler. Very clean samplers may require less than a minute of washing; dirtier samplers may require several minutes of washing.

After washing, the packets are shaken to remove excess water. Next, the packets are cut open and the charcoal is emptied into a new disposable plastic beaker. The beaker has been pre-labeled with the laboratory identification number. The charcoal is now ready for elution. The emptied fiberglass screen packet is discarded. At stations where two or more charcoal packets are collected, one is selected for analysis and the other is frozen and retained until the end of the study. In some studies the analysis protocol stipulates that a fixed percentage (often 5%) of the samples should be duplicates; in these cases the second charcoal packet is separately analyzed. Note that these are duplicate samples, not replicate samples since each packet is, of necessity, placed in a somewhat different location and is therefore exposed to somewhat different conditions.

Cleaning of Glassware

Most of our work uses disposable plastic containers. A small amount of glassware is occasionally used for preparation of standards. It is dedicated to this use. In the event that any glassware does come in contact with tracer dyes it will be carefully cleaned before re-use. To do this cleaning, containers are rinsed several times in clean water. Glassware which may be contaminated with dyes is washed with detergent, and then again rinsed. Next, the glassware is soaked for one hour or more in a bleach and water solution. Upon removal from this soaking, the glassware is rinsed again and allowed to air dry.

Figure 1. Sample Collection Data Sheet

OZARK UNDERGROUND LABORATORY, INC.

1572 Aley Lane Protem, MO 65733 (417) 785-4289 fax (417) 785-4290 email: oul@tri-lakes.net

SAMPLE COLLECTION DATA SHEET for FLUORESCENCE ANALYSIS

Project:_____ Week No:_____ Samples Collected By:_____

Samples Shipped By:_____ Samples Received By:_____

Date Samples Shipped:___/___/___ Date Samples Received:___/___/___ Time Samples Received:____:____ Return Cooler? Yes_____No_____

Bill to:_____ Send Results to: _____

Analyze for: Fluorescein_____Eosine_____Rhodamine WT_____Other_____Ship cooler to: _____

OUL use only		*Please indicate stations where dye was visible in the field for field technician use - use black ink only*						*OUL use only*
# CHAR REC'D	LAB NUMBER	STATION NUMBER 1-4 Numbers	STATION NAME	PLACED		COLLECTED		# WATER REC'D
				DATE	TIME	DATE	TIME	

COMMENTS:_____

This sheet filled out by OUL staff? Yes_____No_____Charts for samples on this page proofed by OUL:_____

Page _____ of _____

Figure 2. Discrepancy Sheet

OZARK UNDERGROUND LABORATORY, INC.

DISCREPANCIES BETWEEN CHAIN-OF-CUSTODY SHEETS AND ACTUAL SAMPLES RECEIVED **Page _____ of _____**

Company & Project Name: **Date Rec'd by OUL:** **Wk # _____**

Lab #	Sta #	Station Name	Date Pulled	Problem	Solution

Comments:

Elution of the Charcoal

There are various eluting solutions which can be used for the recovery of tracer dyes. The solutions typically include an alcohol, some water, and a strong basic solution such as aqueous ammonia.

The standard elution solution now used at the Ozark Underground Laboratory is a mixture of 5% aqua ammonia and 95% isopropyl alcohol solution and sufficient potassium hydroxide flakes to saturate the solution. The isopropyl alcohol is 70% alcohol and 30% water. The aqua ammonia solution is 29% ammonia. The potassium hydroxide is added until a super-saturated layer is visible in the bottom of the container. This super-saturated layer is not used for elution. Preparation of eluting solutions uses dedicated glassware which is never used in contact with dyes or dye solutions.

The eluting solution we use will elute fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. It is also suitable for separating fluorescein peaks from peaks of some naturally present materials found in some samplers.

Fifteen ml of the eluting solution is poured over the washed charcoal in a disposable sample beaker. The sample beaker is capped. The sample is allowed to stand for 60 minutes. After this time, the liquid is carefully poured off the charcoal into a new disposable beaker which has been appropriately labeled with the laboratory identification number. A few grains of charcoal may inadvertently pass into the second beaker; no attempt is made to remove these from the second sample beaker. After the pouring, a small amount of the elutant will remain in the initial sample beaker. After the transfer of the elutant to the second sample beaker, the contents of the first sample beaker (the eluted charcoal) are discarded.

Analysis on the Shimadzu RF-5000U or RF-5301

The Laboratory uses two Shimadzu spectrofluorophotometers. One is a model RF-5000U, and the other is a model RF-5301. Both of these instruments are capable of synchronous scanning. The RF-5301 is the primary instrument used; the RF-5000U is primarily used as a back-up instrument except for tracing studies which were begun using this instrument. The OUL also owns a Shimadzu RF-540 spectrofluorometer which is occasionally used for special purposes.

A sample of the elutant is withdrawn from the sample container using a disposable polyethylene pipette. Approximately 3 ml of the elutant is then placed in disposable rectangular polystyrene cuvette. The cuvette has a maximum capacity of 3.5 ml. The cuvette is designed for fluorometric analysis; all four sides and the bottom are clear. The spectral range of the cuvettes is 340 to 800 nm. The pipettes and cuvettes are discarded after one use.

The cuvette is then placed in the RF-5000U or the RF-5301. Both instruments are controlled by a programmable computer. Each instrument is capable of conducting substantial data analysis.

Our instruments are operated and maintained in accordance with the manufacturer's recommendations. On-site installation of the instruments and a training session on the use of spectrofluorophotometers was provided by Delta Instrument Company.

Our typical analysis of an elutant sample where fluorescein, eosine, rhodamine WT, or sulforhodamine B dyes may be present includes synchronous scanning of excitation and emission spectra with a 17 nm separation between excitation and emission wavelengths. For these dyes, the excitation scan is from 443 to 613 nm; the emission scan is from 460 to 630 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "very fast" on the RF-5000U; it is "fast" on the RF-5301. The typical sensitivity setting used on both instruments is "high."

Our typical analysis of an elutant sample where pyranine dye may be present includes a synchronous scanning of excitation and emission spectra with a 35 nm separation between excitation and emission wavelengths. For this dye, the excitation scan is from 360 to 600 nm; the emission scan is from 395 to 635 nm. The emission fluorescence from the scan is plotted on a graph. The typical scan speed setting is "very fast" on the RF-5000U; it is "fast" on the RF-5301. The typical sensitivity setting on both instruments is "high."

Excitation and emission slit width settings vary between the two instruments. The widths vary with the dyes for which we are sampling and for the matrix in which the dyes may be present. Excitation and emission slit width settings are summarized in Table 1.

Table 1. Excitation and emission slit width settings routinely used for dye analysis. Units are nanometers (nm)

Parameter	**RF5000U**	**RF5301**
Excitation slit for Eos, Fl, RWT, and SRB in elutant	5	3
Emission slit for Eos, Fl, RWT, and SRB in elutant	3	1.5
Excitation slit for Eos, Fl, RWT, and SRB in water	5	5
Emission slit for Eos, Fl, RWT, and SRB in water	10	3
Excitation slit for Pyranine in elutant	5	5
Emission slit for Pyranine in elutant	3	3
Excitation slit for Pyranine in pH adjusted water	5	5
Emission slit for Pyranine in pH adjusted water	3	3

Eos = Eosine. Fl = Fluorescein. RWT = Rhodamine WT. SRB = Sulforhodamine B.

A plot of the synchronous scan for each sample is produced by the instrument; the plot shows emission fluorescence only. It is photocopied as a part of the final record. The synchronous scans are subjected to computer peak picks; peaks are picked to the nearest 0.1 nm. All samples run on the RF-5000U and RF-5301 are stored on disk and printed on normal typing paper with a laser printer; sample information is printed on the chart.

Quantification

We calculate the magnitude of fluorescence peaks for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes. Dye quantities are expressed in microgram per liter (parts per billion; ppb). On the RF-5000U and RF-5301 the dye concentrations are calculated by separating fluorescence peaks due to dyes from background fluorescence on the charts, and then calculating the area within the fluorescence peak. This area is proportional to areas obtained from standard solutions.

Where there are multiple fluorescence peaks it is sometimes necessary to calculate dye concentrations based upon the height of the fluorescence peak rather that the area. The heights of the peaks are also proportional to dye concentrations.

We run dye concentration standards each day the machine is used. Ten separate standards are used; the standard or standards appropriate for the analysis work being conducted are selected. All standards are based upon the as-sold weights of the dyes. The standards are as follows:

1) 10 ppb fluorescein and 100 ppb rhodamine WT in well water from the Jefferson City-Cotter Formation
2) 10 ppb eosine in well water from the Jefferson City-Cotter Formation
3) 100 ppb sulforhodamine B in well water from the Jefferson City-Cotter Formation.
4) 10 ppb pyranine in well water from the Jefferson City-Cotter Formation. A sample of the standard is placed for at least two hours in a high ammonia atmosphere to adjust the pH to a value of 9.5 or greater.
5) 10 ppb fluorescein and 100 ppb rhodamine WT in elutant.
6) 10 ppb eosine in elutant.
7) 100 ppb sulforhodamine B in elutant.
8) 10 ppb pyranine in elutant.

Preparation of Standards

Dye standards are prepared as follows:

Step 1. A small sample of the as-sold dye is placed in a pre-weighed sample vial and the vial is again weighed to determine the weight of the dye. We attempt to use a sample weighing between 1 and 5 grams. This sample is then diluted with well water to make a 1% dye solution by weight (based upon the as-sold weight of the dye). The resulting dye solution is allowed to sit for at least four hours to insure that all dye is fully dissolved.

Step 2. One part of each dye solution from Step 1 is placed in a mixing container with 99 parts of well water. Separate mixtures are made for fluorescein, rhodamine WT, eosine, sulforhodamine B, and pyranine. The resulting solutions contain 100 mg/l dye (100 parts per million dye). The typical prepared volume of this mixture is appropriate for the sample bottles being used; we commonly prepare about 50 ml. of the Step 2 solutions. The dye solution from Step 1 that is used in making the Step 2 solution

is withdrawn with a digital Finnpipette which is capable of measuring volumes between 0.200 and 1.000 ml at intervals of 0.005 ml. The calibration certificate with this instrument indicates that the accuracy (in percent) is as follows:

At 0.200 ml, 0.90%

At 0.300 ml, 0.28%

At 1.000 ml, 0.30%

The Step 2 solution is called the long term standard. Ozark Underground Laboratory experience indicates that Step 2 solutions, if kept refrigerated, will not deteriorate appreciably over periods of less than a year. Furthermore, these Step 2 solutions may last substantially longer than one year.

Step 3. A series of intermediate-term dye solutions are made. Approximately 45 ml. of each intermediate-term dye solution is made. All volume measurements of less than 5 ml are made with a digital Finnpipette. (see description in Step 2). All other volume measurements are made with Rheinland Kohn Geprufte Sicherheit 50 ml. capacity pump dispenser which will pump within plus or minus 1% of the set value. The following solutions are made; all concentrations are based on the as-sold weight of the dyes:

1) A solution containing 1 ppm fluorescein dye and 10 ppm rhodamine WT dye.

2) A solution containing 1 ppm eosine.

3) A solution containing 10 ppm sulforhodamine B dye.

4) A solution containing 1 ppm pyranine.

Step 4. A series of eight short-term dye standards are made from solutions in Step 3. These standards were identified earlier in this section. In the experience of the Ozark Underground Laboratory these standards have a useful shelf life in excess of one week. However, in practice, they are kept under refrigeration and new standards are made weekly.

Dilution of Samples

Some samples contain dye concentrations greater than the analytical instrument can accurately measure. The analysis graph for such a sample is often flat-topped, indicating that it exceeds the measuring scale. In some cases there is a fluorescence peak which looks similar to accurate peaks except that the peak emission wavelength is longer than normally associated with the dye in question. These peaks routinely have arbitrary fluorescence unit values of 800 or more (the maximum value on this arbitrary scale is 1000). All of these samples need to be diluted to permit accurate quantification.

Some water samples have high turbidity or color which interferes with accurate detection and measurement of dye concentrations. It is often possible to dilute these samples and then measure the dye concentration in the diluted sample.

The typical dilution is 100 fold. One part of the test sample is combined with 99 parts of water (if the test sample is water) or with 99 parts of the standard elutant (if the

test sample is elutant). Typically, 0.300 ml of the test solution is combined with 29.700 ml of water (or elutant as appropriate) to yield a new test solution. All volume measurements of less than 5 ml are made with a digital Finnpipette. which is capable of measuring volumes between 0.200 and 1.000 ml at intervals of 0.005 ml. The calibration certificate with this instrument indicates that the accuracy (in percent) is as follows:

At 0.200 ml, 0.90%

At 0.300 ml, 0.28%

At 1.000 ml, 0.30%

All other volume measurements are made with Rheinland Kohn Geprufte Sicherheit 50 ml. capacity pump dispenser which will pump within plus or minus 1% of the set value.

Quality Control

Laboratory blanks are run for every sample where the last two digits of the laboratory numbers are 00, 20, 40, 60, or 80. A charcoal packet is placed in a pumping well sampler and at least 25 gallons of unchlorinated water is passed through the sampler at a rate of about 2.5 gallons per minute. The sampler is then subjected to the same analytical protocol as all other samplers.

System functioning tests of the analytical instruments are conducted in accordance with the manufacturer's recommendations.

All materials used in sampling and analysis work are routinely analyzed for the presence of any compounds which might create fluorescence peaks in or near the acceptable wavelength ranges for any of the tracer dyes. This testing typically includes approximately 1% of materials used.

Reports

Reports are provided in accordance with the needs of the client. At a minimum we provide copies of the analysis graphs and a listing of stations and samples where dye was detected. The reports indicate dye concentrations.

Work at the Ozark Underground Laboratory is directed by Mr. Thomas Aley. Mr. Aley has 36 years of professional experience in hydrology and hydrogeology. He is certified as a Professional Hydrogeologist (Certificate #179) by the American Institute of Hydrology. Mr. Aley has 34 years of professional experience in groundwater tracing with fluorescent tracing agents.

CRITERIA FOR DETERMINATION OF POSITIVE DYE RECOVERIES

Normal Emission Ranges and Detection Limits

The OUL has established normal emission fluorescence wavelength ranges for each of the five dyes. The normal acceptable range equals mean values plus and minus two standard deviations. These values are derived from actual groundwater tracing studies conducted by the OUL.

The detection limits are based upon concentrations of dye necessary to produce emission fluorescence peaks where the signal to noise ratio is 3. The detection limits are realistic for most field studies since they are based upon results from actual field samples rather than being based upon values from spiked samples in a matrix of reagent water or the elutants from unused activated carbon samplers. In some cases detection limits may be smaller than reported if the water being sampled has very little fluorescent material in it. In some cases detection limits may be greater than reported; this most commonly occurs if the sample is turbid due to suspended material or a coloring agent such as tannic compounds. Turbid samples are typically centrifuged or, if this is not effective, diluted prior to analysis.

Table 2 provides normal emission wavelength ranges and detection limits for the five dyes when analyzed on the OUL's RF-5000U spectrofluorophotometer. Table 3 provides similar data for the OUL's RF-5301. As indicated earlier in Table 1, the analytical protocols used on the two instruments are somewhat different, especially in regard to the widths of excitation and emission slit settings.

Table 2. RF-5000U Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	533.0 to 539.6	0.035
Eosine in Water	529.6 to 538.4	0.008
Fluorescein in Elutant	510.7 to 515.0	0.010
Fluorescein in Water	505.6 to 510.5	0.0005
Pyranine in Elutant	500.4 to 504.6	0.055
Pyranine in Water*	501.2 to 505.2	0.030
Rhodamine WT in Elutant	561.7 to 568.9	0.275
Rhodamine WT in Water	569.4 to 574.8	0.050
Sulforhodamine in Elutant	567.5 to 577.5	0.150
Sulforhodamine in Water	576.2 to 579.7	0.040

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Table 3. RF-5301 Spectrofluorophotometer. Normal emission wavelength ranges and detection limits for fluorescein, eosine, rhodamine WT, sulforhodamine B, and pyranine dyes in water and elutant samples. Detection limits are based upon the as-sold weight of the dye mixtures normally used by the OUL.

Dye and Matrix	Normal Acceptable Emission Wavelength Range (nm)	Detection Limit (ppb)
Eosine in Elutant	535.2 to 541.8	0.050
Eosine in Water	532.1 to 540.9	0.015
Fluorescein in Elutant	513.6 to 517.9	0.025
Fluorescein in Water	508.1 to 513.0	0.002
Pyranine in Elutant	502.1 to 508.1	0.015
Pyranine in Water*	504.1 to 510.1	0.010
Rhodamine WT in Elutant	566.6 to 573.8	0.170
Rhodamine WT in Water	574.1 to 579.5	0.015
Sulforhodamine in Elutant	570.8 to 580.8	0.080
Sulforhodamine in Water	579.7 to 583.2	0.008

* pH adjusted water with pH of 9.5 or greater.

Note: The protocols for the analysis of pyranine dye are substantially different than those for the other dyes. As a result, there is less potential interference between pyranine and fluorescein than might otherwise be indicated by the emission wavelength values shown in the table.

Criteria for Determining Positive Dye Recoveries

The following sections identify normal criteria used by the OUL for determining positive dye recoveries. Beginning January 1, 2001, the primary analytical instrument in use at the OUL was the RF-5301; the RF-5000U was the principal backup instrument. Studies which were in progress prior to January 1, 2001 continued to have samples analyzed on the RF-5000U.

Except for pyranine dye, the analytical protocol used for the RF-5301 provides for the use of narrower excitation and/or emission slit settings than the RF-5000U protocol. This enhances our ability to discriminate between dyes and other fluorescent compounds. The protocol which is possible with the RF-5301 (as contrasted with the RF-5000U) also provides for a better balance in the sizes of the fluorescence peaks associated with an equal concentration of all of the dyes.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 535.2 to 541.8 nm for samples analyzed by the RF-5301. The range must be 533.0 to 539.6 nm for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the eosine detection limit in elutant samples is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb. For the RF-5000U the eosine detection limit in elutant samples is 0.035 ppb, thus this dye concentration limit equals 0.105 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of eosine. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of eosine. In addition, there must be no other factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Eosine Dye Recoveries in Water Samples.

There is generally little or no detectable fluorescence background in the general range of eosine dye encountered in most groundwater tracing studies. The following three criteria are used to identify fluorescence peaks which are deemed to be eosine dye.

Criterion 1. The associated charcoal samplers for the station should also contain eosine dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be eosine dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 532.1 to 540.9 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 529.6 to 538.4 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our eosine detection limit in water samples analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb. For samples analyzed on the 5000U the detection limit is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Elutants from Charcoal Samplers.

There is often some fluorescence background in the range of fluorescein dye present at some of the stations used in groundwater tracing studies. We routinely conduct background sampling prior to the introduction of any tracer dyes to characterize this background fluorescence and to identify the existence of any tracer dyes which may be present in the area. The fact that a fluorescence peak is identified in our analytical results is not proof that it is fluorescein dye or that it is fluorescein dye from the trace of concern. The following 4 criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye recoveries from our tracing work.

Criterion 1. There must be at least one fluorescence peak at the station in question in the range of 513.6 to 517.9 nm for samples analyzed by the RF-5301. The range must be 510.7 to 515.0 for samples analyzed by the RF-5000U.

Criterion 2. The dye concentration associated with the fluorescence peak must be at least 3 times the detection limit. For the RF-5301, the fluorescein detection limit in elutant samples is 0.025 ppb, thus this dye concentration limit equals 0.075 ppb. For the RF-5000U, the fluorescein detection limit in elutant samples is 0.010 ppb, thus this dye concentration limit equals 0.030 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of fluorescein. Much background fluorescence yields low, broad, and asymmetrical fluorescence peaks rather than the more narrow and symmetrical fluorescence peaks typical of fluorescein. In addition, there must be no other factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Fluorescein Dye Recoveries in Water Samples.

There is commonly some fluorescence background in the general range of fluorescein dye at some sampling stations used in groundwater tracing studies. The following criteria are used to identify fluorescence peaks which are deemed to be fluorescein dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain fluorescein dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be fluorescein dye from our groundwater tracing work. For samples analyzed on the RF-5301, the fluorescence peak should generally be in the range of 508.1 to 513.0 nm. For samples analyzed on the RF-5000U, the fluorescence peak should generally be in the range of 505.6 to 510.5 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our fluorescein detection limit in water samples analyzed on the RF-5301 is 0.002 ppb, thus this dye concentration limit equals 0.006 ppb. For the RF-5000U the detection limit is 0.0005 ppb, thus this dye concentration limit equals 0.0015 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of Rhodamine WT dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT.

Criterion 1. For samples analyzed on the RF-5301, there must be at least one fluorescence peak at the station in question in the range of 566.6 to 573.8 nm. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 561.7 to 568.9 nm.

Criterion 2. The dye concentration associated with the Rhodamine WT peak must be at least 3 times the detection limit. For the RF-5301, the detection limit in elutant samples is 0.170 ppb, thus this dye concentration limit equals 0.510 ppb. For the RF-5000U, the detection limit in elutant samples is 0.275 ppb, thus this dye concentration limit equals 0.825 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of Rhodamine WT. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Rhodamine WT Dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be Rhodamine WT dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain Rhodamine WT dye in accordance with the criteria listed above. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be Rhodamine WT dye from the tracing work under investigation. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 574.1 to 579.5 nm. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 569.4 to 574.8 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. Our Rhodamine WT detection limit in water samples analyzed on the RF-5301 is 0.015 ppb, thus this dye concentration limit is 0.045 ppb. For samples analyzed on the RF-5000U the detection limit is 0.050 ppb, thus this dye concentration limit equals 0.150 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Sulforhodamine B Dye Recoveries in Elutants from Charcoal Samplers.

There is generally little or no detectable fluorescence background in the general range of sulforhodamine B dye encountered in most groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 567.5 to 577.5 nm. The acceptable range for samples analyzed on the RF-5301 is 570.8 to 580.8 nm.

Criterion 2. The dye concentration associated with the sulforhodamine B peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.150 ppb, thus this dye concentration limit equals 0.450 ppb. For the RF-5301, the detection limit in elutant samples is 0.080 ppb, thus this dye concentration limit equals 0.240 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of sulforhodamine B. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Sulforhodamine B dye Recoveries in Water Samples.

The following criteria are used to identify fluorescence peaks which are deemed to be sulforhodamine B dye in water.

Criterion 1. The associated charcoal samplers for the station should also contain sulforhodamine B dye in accordance with the criteria listed earlier. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be sulforhodamine B dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 576.2 to 579.7 nm. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 579.7 to 583.2 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. For samples analyzed on the RF-5301 the detection limit in water is 0.008 ppb, thus this dye concentration limit equals 0.024 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.040 ppb, thus this dye concentration limit equals 0.120 ppb.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Elutants from Charcoal Samplers.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

There is often some detectable fluorescence background encountered in the general range of pyranine dye in groundwater tracing studies. The following four criteria are used to identify fluorescence peaks which are deemed to be pyranine.

Criterion 1. For samples analyzed on the RF-5000U, there must be at least one fluorescence peak at the station in question in the range of 500.4 to 504.6 nm. The acceptable range for samples analyzed on the RF-5301 is 502.1 to 508.1 nm.

Criterion 2. The dye concentration associated with the pyranine dye peak must be at least 3 times the detection limit. For the RF-5000U, the detection limit in elutant samples is 0.055 ppb, thus this dye concentration limit equals 0.165 ppb. For the RF-5301, the detection limit in elutant samples is 0.015 ppb, thus this dye concentration limit equals 0.045 ppb.

Criterion 3. The dye concentration must be at least 10 times greater than any other concentration reflective of background at the sampling station in question.

Criterion 4. The shape of the fluorescence peak must be typical of pyranine dye. In addition, there must be no other factors which suggest that the fluorescence peak may not be dye from the groundwater tracing work under investigation.

Normal Criteria Used by the Ozark Underground Laboratory for Determining Positive Pyranine Dye Recoveries in Water Samples.

It must be remembered that the analysis protocol for pyranine dye is different than the protocol for the other four dyes discussed in this document. If the other dyes are present in a sample analyzed for pyranine dye their emission fluorescence peaks (if any) will be appreciably different than the values presented above. Because of this, there is very little analytical interference between fluorescein and pyranine dyes when both are present in a sample.

The fluorescence of pyranine decreases below a pH of about 9.5. Prior to analysis water samples are placed in a high ammonia atmosphere for at least two hours. A pyranine dye in water standard is placed in the same atmosphere as the samples. Prior to analysis samples are tested to insure that their pH is 9.5 or greater. If pyranine dye concentrations in a sample are so great as to require dilution for quantification of the dye concentration the diluting water used is OUL reagent water which has been pH adjusted in a high ammonia atmosphere.

The following criteria are used to identify fluorescence peaks which are deemed to be pyranine dye in water.

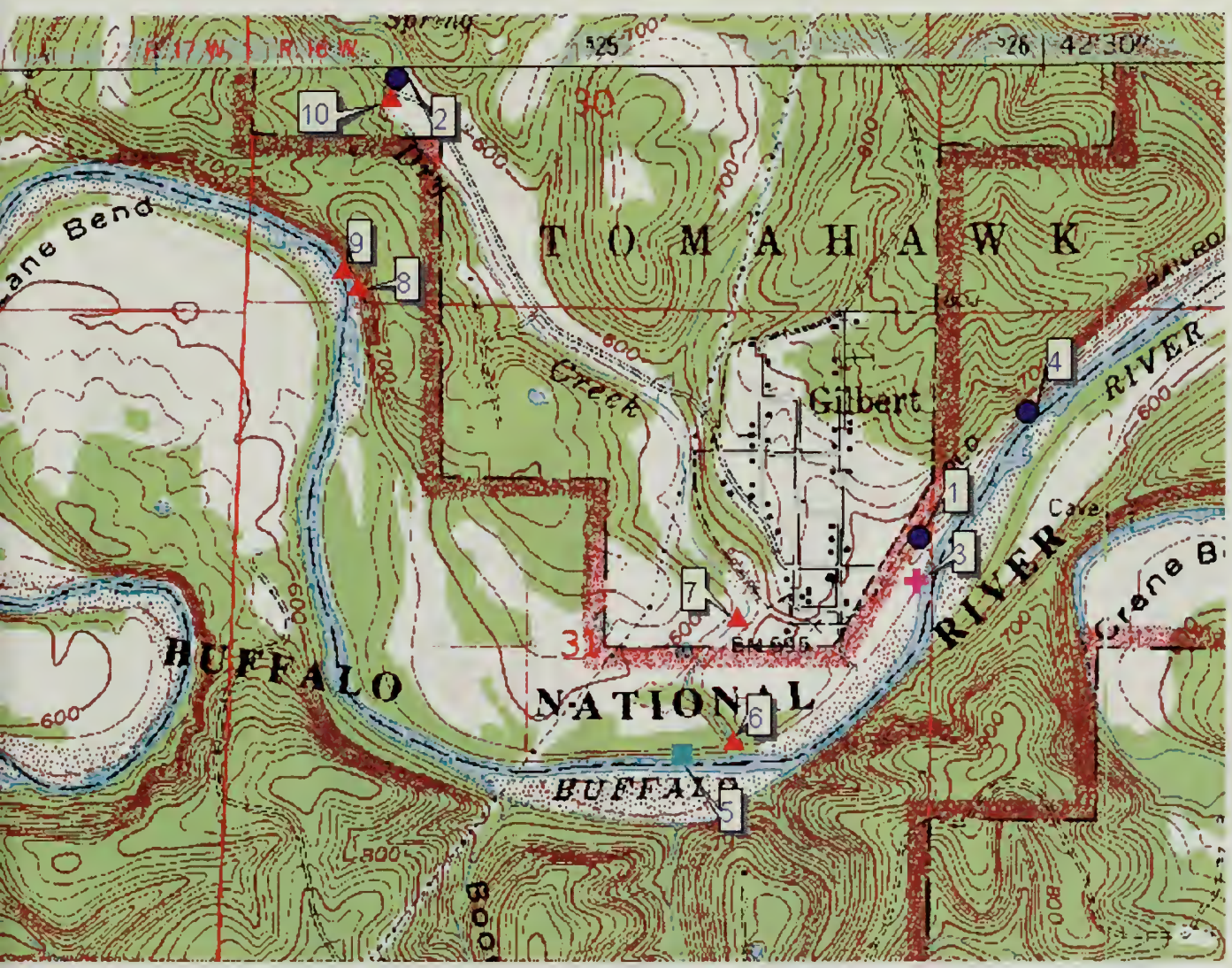
Criterion 1. The associated charcoal samplers for the station should also contain pyranine dye in accordance with the criteria listed earlier. These criteria may be waived if no charcoal sampler exists.

Criterion 2. There must be no factors which suggest that the fluorescence peak may not be pyranine dye from the tracing work under investigation. For samples analyzed with the RF-5000U, the fluorescence peak should generally be in the range of 501.2 to 505.2 nm. For samples analyzed with the RF-5301, the fluorescence peak should generally be in the range of 504.1 to 510.1 nm.

Criterion 3. The dye concentration associated with the fluorescence peak must be at least three times the detection limit. For samples analyzed on the RF-5301 the detection limit in water is 0.010 ppb, thus this dye concentration limit equals 0.030 ppb. For samples analyzed on the RF-5000U the detection limit in water samples is 0.030 ppb, thus this dye concentration limit equals 0.090 ppb.

Appendix B
Maps

Appendix B1 Water Quality/Dye Receptor Sites



0 1000 2000 3000 4000 5000 6000 7000 8000 Feet

Contour Interval = 30

N
W E
S

Water Quality Monitering Site

Routine dye receptor sites

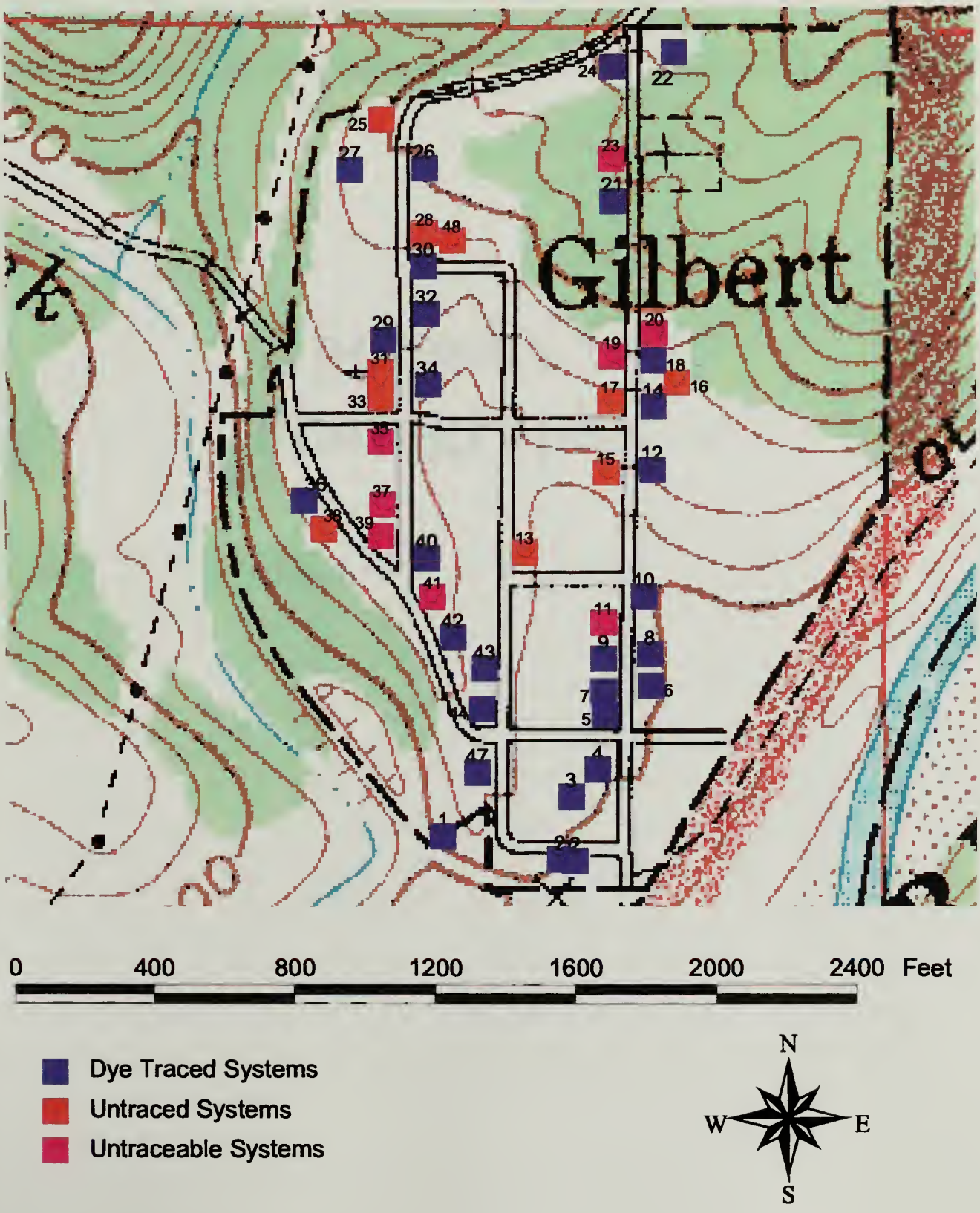
Gilbert Water Quality and Background/Routine Dye Receptor Sites

Background Dye Receptor Sites

Water Quality/Dye Receptor Stations

1= Gilbert Spring
2= Dry Creek
3= Buffalo River upstream of Gilbert Spring
4= Buffalo River downstream of Gilbert Spring
5= Buffalo River upstream of Dry Creek
6= Mouth of Dry Creek
7= Lower Dry Creek Spring
8= Back of Beyond Spring
9= Buffalo River upstream Back of Beyond Spring
10= Spring at HWY 333 Bridge

Appendix B2: Dye Traced Septic Systems in Gilbert



Appendix C
Photographs

Figure C1. Gilbert Spring

Figure C2. Mouth of Gilbert Spring

Figure C3. Losing reach of Dry Creek.

Figure C4. Placement of Dye Receptors.

Figure C5. Injecting Fluorescein dye into Dry Creek for the Time of Travel Trace.

Figure C6. Fluorescein Dye in Dry Creek during Time of Travel Trace.

Figure C7. Flushing fluorescein dye during low risk trace.

Figure C8. Flushing Dye during the low risk trace.

Figure C9 Cistern at site 26

Figure C10 Cistern at site 26

Figure C11. Septic tank at site 26.

Figure C12. Leach field at site 26.

Figure C13. New septic tank at site 26.

Figure C14. Leach field at site 26.

Appendix D
Ozark Underground Laboratory Dye Receptor Results

Gilbert Spring Time of Travel Trace. Water Samples.
February, 2000. Dye introduced at 2/24/2000 at 0915 hours.

Sample	Recovered		Minutes after	Fluorescein Results	
#	Date	Time	Dye Introduction	Peak nm	Conc. ppb
K0133	2/24	1020	65	ND	0
K0134	2/24	1315	240	ND	0
K0135	2/24	1600	405	ND	0
K0136	2/24	1700	465	ND	0
K0137	2/24	1800	525	ND	0
K0138	2/24	1830	555	ND	0
K0139	2/24	1900	585	ND	0
K0141	2/24	1930	615	506.7	0.013
K0142	2/24	2000	645	507.7	0.859
K0143	2/24	2015	660	507.5	4.57
K0144	2/24	2030	675	507.2	9.69
K0145	2/24	2045	690	507.7	17.9
K0146	2/24	2100	705	507.4	32.2
K0147	2/24	2115	720	507.5	56.1
K0148	2/24	2130	735	507.5	83.4
K0149	2/24	2145	750	507.6	117
K0150	2/24	2200	765	507.6	142
K0151	2/24	2215	780	507.7	165
K0152	2/24	2230	795	507.7	183
K0153	2/24	2245	810	507.6	198
K0154	2/24	2300	825	507.6	198
K0155	2/24	2315	840	507.6	200
K0156	2/24	2330	855	507.6	205
K0157	2/24	2345	870	507.5	172
K0158	2/25	0001	886	507.5	158
K0159	2/25	0015	900	507.8	137
K0161	2/25	0030	915	507.8	120
K0162	2/25	0045	930	507.6	108
K0163	2/25	0100	945	507.7	95.6
K0164	2/25	0115	960	507.6	79.0
K0165	2/25	0130	975	507.5	70.4
K0166	2/25	0145	990	507.7	65.4
K0167	2/25	0200	1,005	507.4	54.1
K0168	2/25	0400	1,125	507.6	25.7
K0169	2/25	0600	1,245	507.4	13.6
K0170	2/25	0800	1,365	507.5	8.05
K0171	2/25	1100	1,545	507.7	4.93
K0172	2/25	1230	1,635	507.4	4.11
K0173	2/25	1830	1,995	507.7	2.42
K0174	2/26	1000	2,925	507.6	0.642
K0175	2/26	1745	3,390	507.4	0.246
K0176	2/27	0700	4,185	507.1	0.074

ND = No dye detected

Gilbert Spring Time of Travel Trace. Charcoal Samplers.
February, 2000. Dye introduced at 2/24/2000 at 0915 hours.

Sample	Placed		Recovered		Sampling Period		Fluorescein Results		Mean Conc.
#	Date	Time	Date	Time	Minutes after Dye Introduction		Peak nm	Conc. ppb	per Hr. (ppb)
					Placed	Recovered			
K0119	2/24	1025	2/24	1200	70	165	ND		
K0121	2/24	1200	2/24	1410	165	295	ND		
K0122	2/24	1410	2/24	1600	295	405	ND		
K0123	2/24	1600	2/24	1800	405	525	ND		
K0124	2/24	1800	2/24	2000	525	645	512.5	5.41	2.70
K0125	2/24	2000	2/24	2200	645	765	513.7	4,010	2005
K0126	2/24	2200	2/25	0001	765	886	513.5	12,700	6298
K0127	2/25	0001	2/25	0200	886	1,005	513.8	7,400	3731
K0128	2/25	0200	2/25	0400	1,005	1,125	513.7	2,290	1145
K0129	2/25	0400	2/25	0600	1,125	1,245	513.7	1,150	575
K0130	2/25	0600	2/25	0800	1,245	1,365	513.8	706	353
K0131	2/25	0800	2/25	1100	1,365	1,545	513.7	739	246
K0132	2/25	1100	2/25	1230	1,545	1,635	513.8	226	151

ND = No dye detected

Table 2. **Results for water samples analyzed for the presence of fluorescein, eosine, rhodamine WT (RWT) and sulforhodamine B (SRB) dyes.**
Peak wavelengths are reported in nanometers (nm); dye concentrations are reported in parts per billion (ppb).

OUL #	Stn. #	Station Name	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
				Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
J9718	1	Gilbert Spring	2/1/00 1105	ND		ND		ND		ND	
K0133	1	Gilbert Spring	2/24/00 1020	ND		not analyzed for					
K0134	1	Gilbert Spring	2/24/00 1315	ND							
K0135	1	Gilbert Spring	2/24/00 1600	ND							
K0136	1	Gilbert Spring	2/24/00 1700	ND							
K0137	1	Gilbert Spring	2/24/00 1800	ND							
K0138	1	Gilbert Spring	2/24/00 1830	ND							
K0139	1	Gilbert Spring	2/24/00 1900	ND							
K0141	1	Gilbert Spring	2/24/00 1930	506.7	0.013						
K0142	1	Gilbert Spring	2/24/00 2000	507.7	0.859						
K0143	1	Gilbert Spring	2/24/00 2015	507.5	4.57						
K0144	1	Gilbert Spring	2/24/00 2030	507.2	9.69						
K0145	1	Gilbert Spring	2/24/00 2045	507.7	17.9						
K0146	1	Gilbert Spring	2/24/00 2100	507.4	32.2						
K0147	1	Gilbert Spring	2/24/00 2115	507.5	56.1						
K0148	1	Gilbert Spring	2/24/00 2130	507.5	83.4						
K0149	1	Gilbert Spring	2/24/00 2145	507.6	117						
K0150	1	Gilbert Spring	2/24/00 2200	507.6	142						
K0151	1	Gilbert Spring	2/24/00 2215	507.7	165						
K0152	1	Gilbert Spring	2/24/00 2230	507.7	183						
K0153	1	Gilbert Spring	2/24/00 2245	507.6	198						
K0154	1	Gilbert Spring	2/24/00 2300	507.6	198						
K0155	1	Gilbert Spring	2/24/00 2315	507.6	200						
K0156	1	Gilbert Spring	2/24/00 2330	507.6	205						
K0157	1	Gilbert Spring	2/24/00 2345	507.5	172						
K0158	1	Gilbert Spring	2/25/00 0001	507.5	158						
K0159	1	Gilbert Spring	2/25/00 0015	507.8	137						
K0161	1	Gilbert Spring	2/25/00 0030	507.8	120						
K0162	1	Gilbert Spring	2/25/00 0045	507.6	108						
K0163	1	Gilbert Spring	2/25/00 0100	507.7	95.6						
K0164	1	Gilbert Spring	2/25/00 0115	507.6	79.0						
K0165	1	Gilbert Spring	2/25/00 0130	507.5	70.4						
K0166	1	Gilbert Spring	2/25/00 0145	507.7	65.4						
K0167	1	Gilbert Spring	2/25/00 0200	507.4	54.1						

OUL #	Stn. #	Station Name	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
				Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K0168	1	Gilbert Spring	2/25/00 0400	507.6	25.7						
K0169	1	Gilbert Spring	2/25/00 0600	507.4	13.6						
K0170	1	Gilbert Spring	2/25/00 0800	507.5	8.05						
K0171	1	Gilbert Spring	2/25/00 1100	507.7	4.93						
K0172	1	Gilbert Spring	2/25/00 1230	507.4	4.11						
K0173	1	Gilbert Spring	2/25/00 1830	507.7	2.42						
K0174	1	Gilbert Spring	2/26/00 1000	507.6	0.642						
K0175	1	Gilbert Spring	2/26/00 1745	507.4	0.246						
K0176	1	Gilbert Spring	2/27/00 0700	507.1	0.074						
K0506	1	Gilbert Spring	3/16/00 1015	ND		ND		ND		ND	
K0586	1	Gilbert Spring	3/20/00 1117	507.3	0.041	ND		ND		ND	
K0617	1	Gilbert Spring	3/23/00 0932	506.4	0.025	ND		ND		ND	
K0691	1	Gilbert Spring	3/27/00 1019	507.0	0.040	ND		ND		ND	
K0849	1	Gilbert Spring	3/30/00 0649	506.5	0.011	ND		ND		ND	
K0850	1	Gilbert Spring	4/6/00 1104	505.2 (1)	0.007	ND		ND		ND	
K0927	1	Gilbert Spring	4/11/00 1000	504.8 (1)	0.005	ND		570.2	1.02	ND	
K0928	1	Gilbert Spring	4/13/00 1033	505.6	0.006	ND		570.4	1.87	ND	
K1256	1	Gilbert Spring	4/17/00 1155	505.2 (1)	0.004	ND		570.2	0.618	ND	
K1334	1	Gilbert Spring	4/19/00 2006	505.2 (1)	0.003	ND		570.4	0.746	ND	
K1413	1	Gilbert Spring	4/24/00 1059	ND		ND		567.6 (1)	0.100	ND	
K1454	1	Gilbert Spring	4/27/00 0952	504.4 (1)	0.004	ND		568.0 (1)	0.037	ND	
K1593	1	Gilbert Spring	5/1/00 1025	ND		ND		568.6 (1)	0.063	ND	
K1679	1	Gilbert Spring	5/4/00 1046	ND		ND		566.8 (1)	0.061	ND	
K1781	1	Gilbert Spring	5/8/00 1033	505.6	0.006	ND		569.6	0.730	ND	
K2043	1	Gilbert Spring	5/11/00 1020	ND		ND		569.6 (1)	0.253	ND	
K2044	1	Gilbert Spring	5/15/00 0845	505.2 (1)	0.011	ND		569.2 (1)	0.138	ND	
K2147	1	Gilbert Spring	5/19/00 1409	ND		ND		ND		ND	
K2148	1	Gilbert Spring	5/22/00 1035	505.2 (1)	0.012	ND		568.4 (1)	0.057	ND	
K2213	1	Gilbert Spring	5/25/00 1100	506.0	0.026	ND		568.0 (1)	0.045	ND	
K2324	1	Gilbert Spring	5/30/00 1244	ND		ND		ND		ND	
K2729	1	Gilbert Spring	6/1/00 1020	ND		ND		ND		ND	
K2734	1	Gilbert Spring	6/5/00 0730	507.5	0.042	ND		ND		ND	
K2784	1	Gilbert Spring	6/8/00 0602	504.8 (1)	0.014	ND		ND		ND	
K2785	1	Gilbert Spring	6/12/00 1802	505.6	0.012	ND		ND		ND	
K2806	1	Gilbert Spring	6/15/00 0603	505.6	0.011	ND		ND		ND	
K2883	1	Gilbert Spring	6/20/00 0819	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
				Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K3038	1	Gilbert Spring	6/22/00 1824	ND		ND		ND		ND	
K3039	1	Gilbert Spring	6/29/00 1011	ND		ND		ND		ND	
K3128	1	Gilbert Spring	7/3/00 0920	ND		ND		ND		ND	
K3163	1	Gilbert Spring	7/6/00 0623	505.6	0.009	ND		ND		ND	
K3307	1	Gilbert Spring	7/10/00 1930	ND		ND		ND		ND	
K3325	1	Gilbert Spring	7/13/00 1850	ND		ND		ND		ND	
K3550	1	Gilbert Spring	7/17/00 0915	ND		ND		ND		ND	
K3551	1	Gilbert Spring	7/21/00 1149	ND		ND		ND		ND	
K3705	1	Gilbert Spring	7/25/00 0855	ND		ND		ND		ND	
K3706	1	Gilbert Spring	7/27/00 1447	ND		ND		ND		ND	
J9719	2	Buffalo River u/s of Gilbert Spring	2/1/00 1150	ND		ND		ND		ND	
J9721	4	Lower Dry Creek Spring	2/1/00 1225	ND		ND		ND		ND	
J9722	5	Dry Creek @ Hwy 333	2/1/00 1250	ND		ND		ND		ND	
J9723	6	Back of Beyond Spring	2/1/00 1320	ND		ND		ND		ND	
J9724	7	Buffalo River u/s Back of Beyond Spring	2/1/00 1325	ND		ND		ND		ND	

FOOTNOTES:

ND = No dye detected

NDT = No date/time given

(1) = A fluorescence peak is present that is out of the normally acceptable wavelength range for this dye but has been calculated as a positive dye recovery.

Table 1. Results for charcoal samplers analyzed for the presence of fluorescein, eosine, rhodamine WT (RWT) and sulforhodamine B (SRB) dyes. Peak wavelengths are reported in nanometers (nm); dye concentrations are reported in parts per billion (ppb).

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
J9899	1	Gilbert Spring	2/1/00 1105	2/8/00 1330	ND		ND		ND		ND	
K0035	1	Gilbert Spring	2/8/00 1330	2/15/00 1330	ND		ND		ND		ND	
K0106	1	Gilbert Spring	2/15/00 1330	2/22/00 1240	ND		ND		ND		ND	
K0119	1	Gilbert Spring	2/24/00 1025	2/24/00 1200	ND		Not analyzed for					
K0121	1	Gilbert Spring	2/24/00 1200	2/24/00 1410	ND							
K0122	1	Gilbert Spring	2/24/00 1410	2/24/00 1600	ND							
K0123	1	Gilbert Spring	2/24/00 1600	2/24/00 1800	ND							
K0124	1	Gilbert Spring	2/24/00 1800	2/24/00 2000	512.5	5.41						
K0125	1	Gilbert Spring	2/24/00 2000	2/24/00 2200	513.7	4010						
K0126	1	Gilbert Spring	2/24/00 2200	2/25/00 0001	513.5	12700						
K0127	1	Gilbert Spring	2/25/00 0001	2/25/00 0200	513.8	7400						
K0128	1	Gilbert Spring	2/25/00 0200	2/25/00 0400	513.7	2290						
K0129	1	Gilbert Spring	2/25/00 0400	2/25/00 0600	513.7	1150						
K0130	1	Gilbert Spring	2/25/00 0600	2/25/00 0800	513.8	706						
K0131	1	Gilbert Spring	2/25/00 0800	2/25/00 1100	513.7	739						
K0132	1	Gilbert Spring	2/25/00 1100	2/25/00 1230	513.8	226						
K0177	1	Gilbert Spring	2/22/00 1240	2/28/00 1430	513.8	4,070	ND		ND		ND	
K0177D	1	Gilbert Spring	2/22/00 1240	2/28/00 1430	513.7	4,560	ND		ND		ND	
K0187	1	Gilbert Spring (control)	NDT	2/28/00 1430	513.7	807	ND		ND		ND	
K0328	1	Gilbert Spring (control) q	2/28/00 1430	3/7/00 1252	513.0	30.0	ND		ND		ND	
K0495	1	Gilbert Spring q	3/7/00 1250	3/14/00 1101	512.4	5.08	ND		ND		ND	
K0505	1	Gilbert Spring	3/14/00 1101	3/16/00 1015	511.8	2.02	ND		ND		ND	
K0505D	1	Gilbert Spring	3/14/00 1101	3/16/00 1015	511.3	2.55	ND		ND		ND	
K0583	1	Gilbert Spring	3/16/00 1115	3/20/00 1117	512.6	45.4	ND		ND		ND	
K0614	1	Gilbert Spring	3/20/00 1117	3/23/00 0932	513.0	17.5	ND		ND		ND	
K0688	1	Gilbert Spring q	3/23/00 0932	3/27/00 1019	512.8	27.6	ND		ND		ND	
K0837	1	Gilbert Spring q	3/27/00 0932	3/30/00 0649	512.5	20.0	ND		ND		ND	
K0837D	1	Gilbert Spring q	3/27/00 0932	3/30/00 0649	512.6	14.5	ND		ND		ND	
K0841	1	Gilbert Spring	3/30/00 0649	4/3/00 1530	512.9	20.7	ND		ND		ND	
K0845	1	Gilbert Spring	4/3/00 1530	4/6/00 1104	512.3	11.7	ND		ND		ND	
K0918	1	Gilbert Spring	4/6/00 1104	4/11/00 1000	512.6	14.7	ND		564.2	774	ND	
K0923	1	Gilbert Spring	4/11/00 1000	4/13/00 1033	512.3	10.2	ND		564.6	301	ND	
K1253	1	Gilbert Spring	4/13/00 1033	4/17/00 1155	512.5	13.8	ND		563.8	281	ND	
K1330	1	Gilbert Spring	4/17/00 1155	4/19/00 2006	512.6	5.66	ND		563.5	201	ND	
K1330D	1	Gilbert Spring	4/17/00 1155	4/19/00 2006	512.2	6.89	ND		563.8	207	ND	
K1409	1	Gilbert Spring	4/19/00 2006	4/24/00 1059	512.3	13.3	ND		563.8	159	ND	
K1450	1	Gilbert Spring	4/24/00 1059	4/27/00 0952	512.6	7.14	ND		563.0	26.2	ND	
K1589	1	Gilbert Spring	4/27/00 0952	5/1/00 1025	512.6	11.9	ND		562.8	27.0	ND	
K1675	1	Gilbert Spring	5/1/00 1025	5/4/00 1046	512.2	6.29	ND		563.1	44.4	ND	
K1776	1	Gilbert Spring	5/4/00 1046	5/8/00 1033	512.2	11.7	ND		564.5	736	ND	

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OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K2034	1	Gilbert Spring	5/8/00 1033	5/11/00 1020	512.2	6.42	ND		563.8	120	ND	
K2038	1	Gilbert Spring	5/11/00 1020	5/15/00 0845	512.2	8.93	ND		563.5	70.9	ND	
K2038D	1	Gilbert Spring	5/11/00 1020	5/15/00 0845	511.8	7.39	ND		563.3	45.6	ND	
K2137	1	Gilbert Spring	5/15 0845	5/19/00 1409	512.5	10.9	ND		562.8	98.6	ND	
K2137D	1	Gilbert Spring	5/15 0845	5/19/00 1409	512.2	10.5	ND		563.4	89.7	ND	
K2143	1	Gilbert Spring	5/19 1409	5/22/00 1035	512.2	11.5	ND		562.2	24.3	ND	
K2209	1	Gilbert Spring	5/22/00 1035	5/25/00 1100	512.3	7.95	ND		561.9	28.6	ND	
K2319	1	Gilbert Spring	5/25/00 1100	5/30/00 1244	512.3	8.44	ND		561.8	20.7	ND	
K2725	1	Gilbert Spring	5/30/00 1244	6/1/00 1020	510.0 (2)	2.23	ND		565.2	1.39	ND	
K2725D	1	Gilbert Spring	5/30/00 1244	6/1/00 1020	510.9	2.32	ND		564.0	1.62	ND	
K2730	1	Gilbert Spring	6/1/00 1020	6/5/00 0730	512.2	8.50	ND		563.2	1.38	ND	
K2775	1	Gilbert Spring	NDT	6/8/00 0602	512.5	11.4	ND		562.0	3.22	ND	
K2779	1	Gilbert Spring	NDT	6/12/00 1802	512.5	24.0	ND		562.0	4.70	ND	
K2803	1	Gilbert Spring	06/12 NT	6/15/00 0603	512.4	8.91	ND		562.0	12.6	ND	
K2881	1	Gilbert Spring	6/15/00 0603	6/20/00 0819	511.8	4.75	ND		ND		ND	
K2881D	1	Gilbert Spring	6/15/00 0603	6/20/00 0819	512.2	4.76	ND		ND		ND	
K3034	1	Gilbert Spring	06/19 NT	6/22/00 1824	511.1	3.41	ND		ND		ND	
K3036	1	Gilbert Spring	6/22/00 1824	6/29/00 1011	512.0	5.79	ND		ND		ND	
K3125	1	Gilbert Spring	6/29/00 1011	7/3/00 0920	511.4	6.21	ND		ND		ND	
K3158	1	Gilbert Spring	7/3/00 0920	7/6/00 0623	512.1	7.46	ND		ND		ND	
K3158D	1	Gilbert Spring	7/3/00 0920	7/6/00 0623	511.6	7.42	ND		ND		ND	
K3303	1	Gilbert Spring	7/6/00 0623	7/10/00 1930	511.2	11.2	ND		ND		ND	
K3321	1	Gilbert Spring	7/10/00 1930	7/13/00 1850	511.4	3.27	ND		ND		ND	
K3542	1	Gilbert Spring	7/13/00 1850	7/17/00 0915	511.7	6.50	ND		ND		ND	
K3546	1	Gilbert Spring	7/17/00 0915	7/21/00 1149	511.8	5.30	ND		ND		ND	
K3696	1	Gilbert Spring	7/21/00 1149	7/25/00 0855	511.8	5.33	ND		ND		ND	
K3701	1	Gilbert Spring q	7/25/00 0855	7/27/00 1447	511.4	3.02	ND		ND		ND	
K7043	1	Gilbert Spring q	11/17/00 1630	11/27/00 1700	510.8 s	2.38	ND		ND		ND	
K7044	1	Gilbert Spring	11/27/00 1700	12/5/00 1200	ND		ND		ND		ND	
K7418	1	Gilbert Spring	12/5/00 1200	12/19/00 1245	510.8 s	2.49	ND		ND		ND	
K7643	1	Gilbert Spring	12/19/00 1245	1/2/01 1210	510.8 s	1.72	ND		ND		ND	
K7758	1	Gilbert Spring	1/2/01 1210	1/5/01 1610	510.7 s	1.92	ND		ND		ND	
K7759	1	Gilbert Spring	1/5/01 1610	1/9/01 1030	513.4	449	ND		ND		ND	
K8006	1	Gilbert Spring	1/9/01 1030	1/12/01 1250	513.8	195	ND		564.4	SH	ND	
K8006D	1	Gilbert Spring	1/9/01 1030	1/12/01 1250	514.0	223	ND		564.8	SH	ND	
K8007	1	Gilbert Spring	1/12/01 1250	1/16/01 1250	513.0	75.9	ND		562.0	4.65	ND	
K8053	1	Gilbert Spring	1/16/01 1250	1/19/01 0820	512.6	37.7	ND		562.8	14.8	ND	
K8054	1	Gilbert Spring	1/19/01 0820	1/23/01 1630	512.5	29.9	ND		562.2	12.9	ND	
K8150	1	Gilbert Spring	1/23/01 1630	1/26/01 0915	512.7	40.6	ND		561.8	7.90	ND	
K8151	1	Gilbert Spring	1/26/01 0915	1/30/01 1120	512.9	62.2	ND		562.0	9.31	ND	
J9901	2	Buffalo River u/s of Gilbert Spring	2/1/00 1150	2/8/00 1350	ND		ND		ND		ND	
K0036	2	Buffalo River u/s of Gilbert Spring	2/8/00 1350	2/15/00 1245	ND		ND		ND		ND	
K0107	2	Buffalo River u/s of Gilbert Spring	2/15/00 1245	2/22/00 1300	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K0178	2	Buffalo River u/s of Gilbert Spring	2/22/00 1300	2/28/00 1200	511.1	1.84	ND		ND		ND	
K0321	2	Buffalo River u/s of Gilbert Spring	2/28/00 1200	3/7/00 1240	510.8 (1)	1.52	ND		ND		ND	
K0496	2	Buffalo River u/s of Gilbert Spring	3/7/00 1240	3/14/00 1023	ND		539.6	SH	ND		ND	
K0584	2	Buffalo River u/s of Gilbert Spring	3/14/00 1023	3/20/00 1100	ND		ND		ND		ND	
K0615	2	Buffalo River u/s of Gilbert Spring	3/20/00 1100	3/23/00 0923	ND		539.6	SH	ND		ND	
K0689	2	Buffalo River u/s of Gilbert Spring q	3/23/00 0923	3/27/00 1005	ND		538.8	SH	ND		ND	
K0838	2	Buffalo River u/s of Gilbert Spring q	3/27/00 0923	3/30/00 0657	ND		536.0	SH	ND		ND	
K0842	2	Buffalo River u/s of Gilbert Spring	3/30/00 0657	4/3/00 1545	ND		537.6	SH	ND		ND	
K0846	2	Buffalo River u/s of Gilbert Spring	4/3/00 1545	4/6/00 1040	ND		ND		ND		ND	
K0919	2	Buffalo River u/s of Gilbert Spring	4/6/00 1040	4/11/00 0922	ND		536.8	SH	ND		ND	
K0924	2	Buffalo River u/s of Gilbert Spring	4/11/00 0922	4/13/00 0957	ND		537.2	SH	ND		ND	
	2	Buffalo River u/s of Gilbert Spring	4/13/00 0957	4/17/00 1025	(3)							
K1331	2	Buffalo River u/s of Gilbert Spring	4/17/00 1025	4/19/00 2012	ND		ND		ND		ND	
K1410	2	Buffalo River u/s of Gilbert Spring	4/19/00 2012	4/24/00 1115	ND		538.0	SH	ND		ND	
K1451	2	Buffalo River u/s of Gilbert Spring	4/24/00 1115	4/27/00 0923	ND		539.6	SH	ND		ND	
K1590	2	Buffalo River u/s of Gilbert Spring	4/27/00 0923	5/1/00 1000	ND		538.0	SH	ND		ND	
K1676	2	Buffalo River u/s of Gilbert Spring	5/1/00 1000	5/4/00 1115	ND		ND		ND		ND	
K1777	2	Buffalo River u/s of Gilbert Spring	5/4/00 1115	5/8/00 1039	ND		539.2	SH	ND		ND	
K2142	2	Buffalo River u/s of Gilbert Spring q	NDT	5/11/00 1030	ND		ND		ND		ND	
K2039	2	Buffalo River u/s of Gilbert Spring q	5/11/00 1033	5/15/00 0926	ND		ND		ND		ND	
K2138	2	Buffalo River u/s of Gilbert Spring	5/15 0926	5/19/00 1420	ND		ND		ND		ND	
K2144	2	Buffalo River u/s of Gilbert Spring	5/19 1420	5/22/00 1222	ND		ND		ND		ND	
K2210	2	Buffalo River u/s of Gilbert Spring	5/22/00 1222	5/25/00 1113	ND		ND		ND		ND	
K2321	2	Buffalo River u/s of Gilbert Spring	5/25/00 1113	5/30/00 1310	ND		ND		ND		ND	
K2726	2	Buffalo River u/s of Gilbert Spring	5/30/00 1310	6/1/00 1032	ND		ND		ND		ND	
K2731	2	Buffalo River u/s of Gilbert Spring	6/1/00 1032	6/5/00 0739	ND		ND		ND		ND	
K2776	2	Buffalo River u/s of Gilbert Spring	NDT	6/8/00 0612	ND		ND		ND		ND	
K2781	2	Buffalo River u/s of Gilbert Spring	NDT	6/12/00 1816	ND		ND		ND		ND	
	2	Buffalo River u/s of Gilbert Spring	6/12/00 1816	6/29/00 1020	(3)							
K3126	2	Buffalo River u/s of Gilbert Spring	6/29/00 1020	7/3/00 0932	ND		ND		ND		ND	
K3159	2	Buffalo River u/s of Gilbert Spring	7/3/00 0932	7/6/00 0634	ND		ND		ND		ND	
K3304	2	Buffalo River u/s of Gilbert Spring	7/6/00 0634	7/10/00 1942	ND		ND		ND		ND	
K3322	2	Buffalo River u/s of Gilbert Spring	7/10/00 1942	7/13/00 1858	ND		ND		ND		ND	
K3543	2	Buffalo River u/s of Gilbert Spring	7/13/00 1858	7/17/00 0757	ND		538.8	SH	ND		ND	
K3547	2	Buffalo River u/s of Gilbert Spring	7/17/00 0757	7/21/00 1107	ND		ND		ND		ND	
K3697	2	Buffalo River u/s of Gilbert Spring	7/21/00 1107	7/25/00 0938	ND		ND		ND		ND	
K3702	2	Buffalo River u/s of Gilbert Spring	7/25/00 0938	7/27/00 1441	ND		ND		ND		ND	
J9902	**3**	**Mouth of Dry Creek**	**2/1/00 1215**	**2/8/00 1415**	**ND**		**ND**		**ND**		**ND**	
K0037	3	Mouth of Dry Creek	2/8/00 1415	2/15/00 1305	ND		ND		ND		ND	
K0108	3	Mouth of Dry Creek	2/15/00 1305	2/22/00 1325	ND		ND		ND		ND	
K0179	3	Mouth of Dry Creek	2/22/00 1325	2/28/00 1222	ND		ND		ND		ND	
K0322	3	Mouth of Dry Creek	2/28/00 1222	3/7/00 1225	ND		ND		ND		ND	
K0497	3	Mouth of Dry Creek	3/7/00 1225	3/14/00 1030	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
J9903	**4**	**Lower Dry Creek Spring**	**2/1/00 1225**	**2/8/00 1420**	ND		ND		ND		ND	
K0038	4	Lower Dry Creek Spring	2/8/00 1420	2/15/00 1315	ND		ND		ND		ND	
K0109	4	Lower Dry Creek Spring	2/15/00 1315	2/22/00 1335	ND		ND		ND		ND	
K0109D	4	Lower Dry Creek Spring	2/15/00 1315	2/22/00 1335	ND		ND		ND		ND	
K0181	4	Lower Dry Creek Spring	2/22/00 1335	2/28/00 1232	ND		ND		ND		ND	
K0323	4	Lower Dry Creek Spring	2/28/00 1232	3/7/00 1205	ND		ND		ND		ND	
K0323D	4	Lower Dry Creek Spring	2/28/00 1232	3/7/00 1205	ND		ND		ND		ND	
K0498	4	Lower Dry Creek Spring	3/7/00 1205	3/14/00 1039	ND		ND		ND		ND	
J9904	**5**	**Dry Creek @ Hwy 333**	**2/1/00 1250**	**2/8/00 1445**	ND		ND		ND		ND	
K0039	5	Dry Creek @ Hwy 333	2/8/00 1445	2/15/00 1345	ND		ND		ND		ND	
K0110	5	Dry Creek @ Hwy 333	2/15/00 1345	2/22/00 1420	ND		ND		ND		ND	
K0182	5	Dry Creek @ Hwy 333	2/22/00 1420	2/28/00 1325	ND		ND		ND		ND	
K0324	5	Dry Creek @ Hwy 333	2/28/00 1325	3/7/00 1150	ND		ND		ND		ND	
K0499	5	Dry Creek @ Hwy 333	3/7/00 1150	3/14/00 0939	ND		ND		ND		ND	
K0585	5	Dry Creek @ Hwy 333	3/14/00 0939	3/20/00 1051	ND		ND		ND		ND	
K0616	5	Dry Creek @ Hwy 333	3/20/00 1051	3/23/00 0915	ND		ND		ND		ND	
K0690	5	Dry Creek @ Hwy 333 q	3/23/00 0915	3/27/00 0956	ND		ND		ND		ND	
K0839	5	Dry Creek @ Hwy 333 q	3/27/00 0915	3/30/00 0707	ND		ND		ND		ND	
K0843	5	Dry Creek @ Hwy 333	3/30/00 0707	4/3/00 1600	ND		ND		ND		ND	
K0847	5	Dry Creek @ Hwy 333	4/3/00 1600	4/6/00 1030	ND		ND		ND		ND	
K0921	5	Dry Creek @ Hwy 333	4/6/00 1030	4/11/00 0823	ND		537.2	SH	ND		ND	
K0921D	5	Dry Creek @ Hwy 333	4/6/00 1030	4/11/00 0823	ND		536.0	SH	ND		ND	
K0925	5	Dry Creek @ Hwy 333	4/11/00 0823	4/13/00 0950	ND		ND		ND		ND	
K1254	5	Dry Creek @ Hwy 333	4/13/00 0950	4/17/00 1007	ND		ND		ND		ND	
K1332	5	Dry Creek @ Hwy 333	4/17/00 1007	4/19/00 2020	ND		ND		ND		ND	
K1411	5	Dry Creek @ Hwy 333	4/19/00 2020	4/24/00 1124	ND		ND		ND		ND	
K1452	5	Dry Creek @ Hwy 333	4/24/00 1124	4/27/00 0914	ND		539.2	SH	ND		ND	
K1591	5	Dry Creek @ Hwy 333	4/27/00 0914	5/1/00 1043	ND		ND		ND		ND	
K1677	5	Dry Creek @ Hwy 333	5/1/00 1043	5/4/00 0935	ND		ND		ND		ND	
K1778	5	Dry Creek @ Hwy 333	5/4/00 0935	5/8/00 1010	ND		ND		ND		ND	
K2035	5	Dry Creek @ Hwy 333	5/8/00 1010	5/11/00 1010	ND		ND		ND		ND	
K2041	5	Dry Creek @ Hwy 333	5/11/00 1010	5/15/00 0750	ND		ND		ND		ND	
K2139	5	Dry Creek @ Hwy 333	5/15 0750	5/19/00 1403	ND		ND		ND		ND	
K2145	5	Dry Creek @ Hwy 333	5/19 1403	5/22/00 0958	ND		ND		ND		ND	
K2211	5	Dry Creek @ Hwy 333	5/22/00 0958	5/25/00 1145	ND		ND		ND		ND	
K2322	5	Dry Creek @ Hwy 333	5/25/00 1145	5/30/00 1235	ND		ND		ND		ND	
K2727	5	Dry Creek @ Hwy 333	5/30/00 1235	6/1/00 1014	ND		ND		ND		ND	
K2732	5	Dry Creek @ Hwy 333	6/1/00 1014	6/5/00 0724	ND		ND		ND		ND	
K2777	5	Dry Creek @ Hwy 333	NDT	6/8/00 0639	ND		ND		ND		ND	
K2782	5	Dry Creek @ Hwy 333	NDT	6/12/00 1755	ND		ND		ND		ND	
K2804	5	Dry Creek @ Hwy 333	06/12 NT	6/15/00 0556	ND		ND		ND		ND	
K2882	5	Dry Creek @ Hwy 333 q	6/15/00 0556	6/20/00 0812	ND		ND		ND		ND	
K3035	5	Dry Creek @ Hwy 333 q	06/19 NT	6/22/00 1813	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Resuits		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K3037	5	Dry Creek @ Hwy 333	6/22/00 1813	6/29/00 1001	ND		ND		ND		ND	
K3127	5	Dry Creek @ Hwy 333	6/29/00 1001	7/3/00 0905	ND		ND		ND		ND	
K3161	5	Dry Creek @ Hwy 333	7/3/00 0905	7/6/00 0615	ND		ND		ND		ND	
K3305	5	Dry Creek @ Hwy 333	7/6/00 0615	7/10/00 2030	ND		ND		ND		ND	
K3323	5	Dry Creek @ Hwy 333	7/10/00 2030	7/13/00 1845	ND		ND		ND		ND	
K3544	5	Dry Creek @ Hwy 333	7/13/00 1845	7/17/00 1028	ND		ND		ND		ND	
K3548	5	Dry Creek @ Hwy 333	7/17/00 1028	7/21/00 1238	ND		ND		ND		ND	
K3698	5	Dry Creek @ Hwy 333	7/21/00 1238	7/25/00 0835	ND		ND		ND		ND	
K3703	5	Dry Creek @ Hwy 333	7/25/00 0835	7/27/00 1441	ND		ND		ND		ND	
J9905	**6**	**Back of Beyond Spring**	**2/1/00 1320**	**2/8/00 1545**	**ND**		**ND**		**ND**		**ND**	
K0041	6	Back of Beyond Spring	2/8/00 1545	2/15/00 1410	ND		ND		ND		ND	
K0115	6	Back of Beyond Spring	2/15/00 1410	2/22/00 1515	ND		ND		ND		ND	
K0183	6	Back of Beyond Spring	2/22/00 1515	2/28/00 1300	ND		ND		ND		ND	
K0325	6	Back of Beyond Spring q	2/28/00 1300	3/7/00 1120	ND		ND		ND		ND	
K0329	6	Back of Beyond Spring q	2/22/00 1515	3/7/00 1135	ND		ND		ND		ND	
K0501	6	Back of Beyond Spring	3/7/00 1120	3/14/00 0922	ND		ND		ND		ND	
J9906	**7**	**Buffalo River u/s Back of Beyond Spring**	**2/1/00 1325**	**2/8/00 1530**	**ND**		**ND**		**ND**		**ND**	
K0042	7	Buffalo River u/s Back of Beyond Spring	2/8/00 1530	2/15/00 1405	ND		536.0	SH	ND		ND	
K0111	7	Buffalo River u/s Back of Beyond Spring	2/15/00 1405	2/22/00 1502	ND		ND		ND		ND	
K0184	7	Buffalo River u/s Back of Beyond Spring q	2/22/00 1502	2/28/00 1305	ND		ND		ND		ND	
K0330	7	Buffalo River u/s Back of Beyond Spring q	2/22/00 1502	3/7/00 1130	ND		ND		ND		ND	
K0502	7	Buffalo River u/s Back of Beyond Spring	3/7/00 1130	3/14/00 0930	ND		ND		ND		ND	
K0043	**8**	**Buffalo River u/s Dry Creek**	**2/8/00 1410**	**2/15/00 1255**	**ND**		**ND**		**ND**		**ND**	
K0043D	8	Buffalo River u/s Dry Creek	2/8/00 1410	2/15/00 1255	ND		ND		ND		ND	
K0112	8	Buffalo River u/s Dry Creek	2/15/00 1255	2/22/00 1315	ND		ND		ND		ND	
K0185	8	Buffalo River u/s Dry Creek	2/22/00 1315	2/28/00 1216	ND		ND		ND		ND	
K0326	8	Buffalo River u/s Dry Creek q	2/28/00 1216	3/7/00 1220	ND		ND		ND		ND	
K0331	8	Buffalo River u/s Dry Creek q	2/22/00 1315	3/7/00 1220	ND		ND		ND		ND	
K0503	8	Buffalo River u/s Dry Creek	3/7/00 1220	3/14/00 1047	ND		ND		ND		ND	
K0044	**9**	**Small Spring d/s 333 Bridge**	**2/8/00 1500**	**2/15/00 1350**	**ND**		**ND**		**ND**		**ND**	
K0113	9	Small Spring d/s 333 Bridge	2/15/00 1350	2/22/00 1445	ND		ND		ND		ND	
K0186	9	Small Spring d/s 333 Bridge	2/22/00 1445	2/28/00 1345	ND		ND		ND		ND	
K0327	9	Small Spring d/s 333 Bridge	2/28/00 1345	3/7/00 1155	ND		ND		ND		ND	
K0504	9	Small Spring d/s 333 Bridge	3/7/00 1155	3/14/00 1008	ND		ND		ND		ND	
K0114	**10**	**Back of Beyond Spring @ River**	NDT	**2/22/00 1510**	**ND**		**ND**		**ND**		**ND**	
K0844	**12**	**Buffalo River d/s Gilbert Spring**	**3/31/00 1400**	**4/3/00 1520**	**ND**		**ND**		**ND**		**ND**	
K0848	12	Buffalo River d/s Gilbert Spring	4/3/00 1520	4/6/00 1053	ND		ND		ND		ND	
K0922	12	Buffalo River d/s Gilbert Spring	4/6/00 1053	4/11/00 0935	ND		ND		ND		ND	
K0926	12	Buffalo River d/s Gilbert Spring	4/11/00 0935	4/13/00 1022	ND		ND		ND		ND	
K1255	12	Buffalo River d/s Gilbert Spring	4/13/00 1022	4/17/00 1142	ND		ND		ND		ND	
K1333	12	Buffalo River d/s Gilbert Spring	4/17/00 1142	4/19/00 2000	ND		ND		ND		ND	
K1412	12	Buffalo River d/s Gilbert Spring	4/19/00 2000	4/24/00 1046	ND		ND		ND		ND	
K1453	12	Buffalo River d/s Gilbert Spring	4/24/00 1046	4/27/00 0933	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb
K1592	12	Buffalo River d/s Gilbert Spring	4/27/00 0933	5/1/00 1010	ND		ND		ND		ND	
K1678	12	Buffalo River d/s Gilbert Spring	5/1/00 1010	5/4/00 1126	ND		ND		ND		ND	
K1779	12	Buffalo River d/s Gilbert Spring	5/4/00 1126	5/8/00 1022	ND		ND		ND		ND	
K2036	12	Buffalo River d/s Gilbert Spring q	5/8/00 1022	5/11/00 1042	ND		ND		ND		ND	
K2037	12	Buffalo River d/s Gilbert Spring q	5/4 NT	5/11/00 1042	ND		ND		ND		ND	
K2042	12	Buffalo River d/s Gilbert Spring	5/11/00 1042	5/15/00 0935	ND		ND		ND		ND	
K2141	12	Buffalo River d/s Gilbert Spring	5/15 0935	5/19/00 1432	ND		ND		ND		ND	
K2146	12	Buffalo River d/s Gilbert Spring	5/19 1432	5/22/00 1203	ND		ND		ND		ND	
K2212	12	Buffalo River d/s Gilbert Spring	5/22/00 1203	5/25/00 1125	ND		ND		ND		ND	
K2323	12	Buffalo River d/s Gilbert Spring	5/25/00 1125	5/30/00 1325	ND		ND		ND		ND	
K2728	12	Buffalo River d/s Gilbert Spring	5/30/00 1325	6/1/00 1046	ND		ND		ND		ND	
K2733	12	Buffalo River d/s Gilbert Spring	6/1/00 1046	6/5/00 0754	ND		ND		ND		ND	
K2778	12	Buffalo River d/s Gilbert Spring	NDT	6/8/00 0622	ND		ND		ND		ND	
K2783	12	Buffalo River d/s Gilbert Spring	NDT	6/12/00 1830	ND		ND		ND		ND	
K2805	12	Buffalo River d/s Gilbert Spring	06/12 NT	6/15/00 0614	ND		ND		ND		ND	
	12	Buffalo River d/s Gilbert Spring	6/15/00 0614	7/3/00 0950	(3)							
K3162	12	Buffalo River d/s Gilbert Spring	7/3/00 0950	7/6/00 0650	ND		ND		ND		ND	
K3306	12	Buffalo River d/s Gilbert Spring	7/6/00 0650	7/10/00 1951	ND		ND		ND		ND	
K3324	12	Buffalo River d/s Gilbert Spring	7/10/00 1951	7/13/00 1905	ND		ND		ND		ND	
K3545	12	Buffalo River d/s Gilbert Spring	7/13/00 1905	7/17/00 0826	ND		ND		ND		ND	
K3549	12	Buffalo River d/s Gilbert Spring	7/17/00 0826	7/21/00 1117	ND		ND		ND		ND	
K3699	12	Buffalo River d/s Gilbert Spring	7/21/00 1117	7/25/00 1001	ND		ND		ND		ND	
K3704	12	Buffalo River d/s Gilbert Spring	7/25/00 1001	7/27/00 1500	ND		ND		ND		ND	

OUL #	Stn. #	Station Name	Date/Time Placed	Date/Time Collected	Fluorescein Results		Eosine Results		RWT Results		SRB Results	
					Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb	Peak nm	Conc. ppb

FOOTNOTES:

ND = No dye detected

NDT = No date/time given

(1) = A fluorescence peak is present that is atypical in shape but in the normally acceptable wavelength range for this dye and has been calculated as a positive dye recovery.

(2) = A fluorescence peak is present that is out of the normally acceptable wavelength range for this dye but has been calculated as a positive dye recovery.

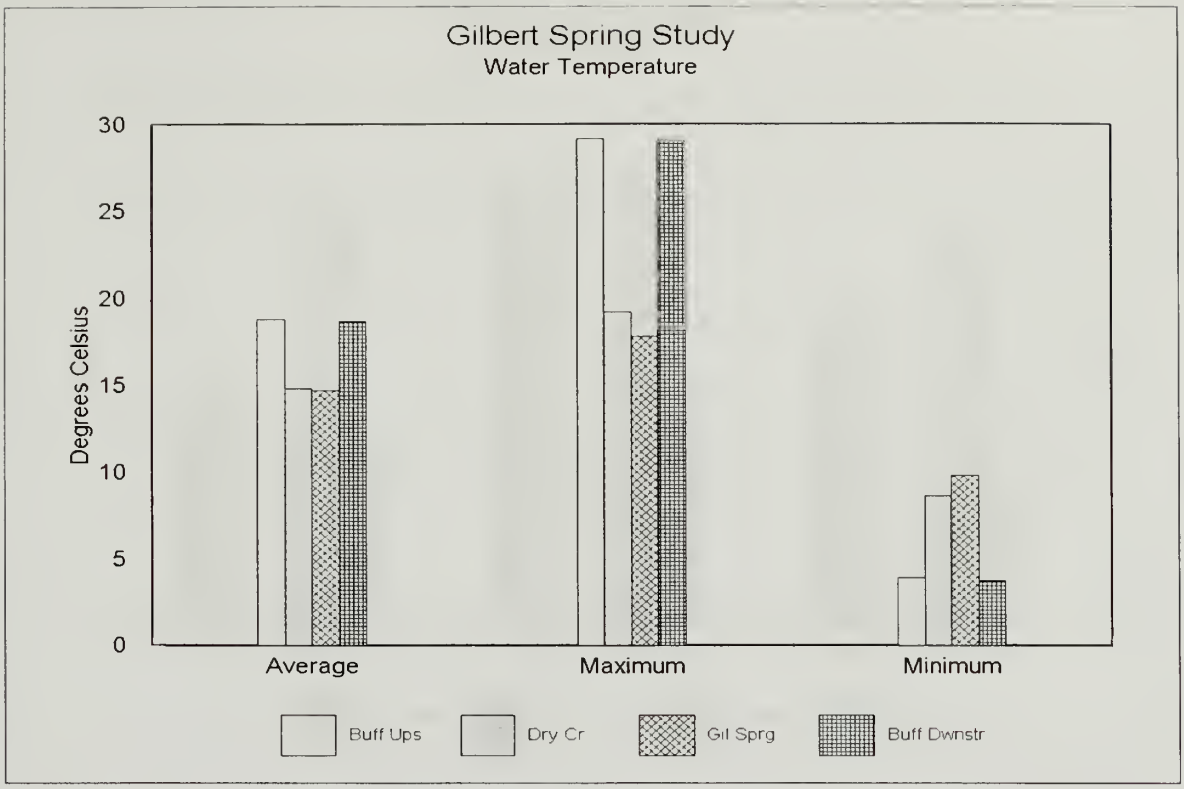
SH = Shoulder. In order to definitively conclude that the fluorescence in a sample is a given dye, it has to fall within the normally acceptable wavelength range of the dye, equal or exceed the detection limit of the dye, and it has to have a definite peak. A shoulder does not meet all of these criteria.

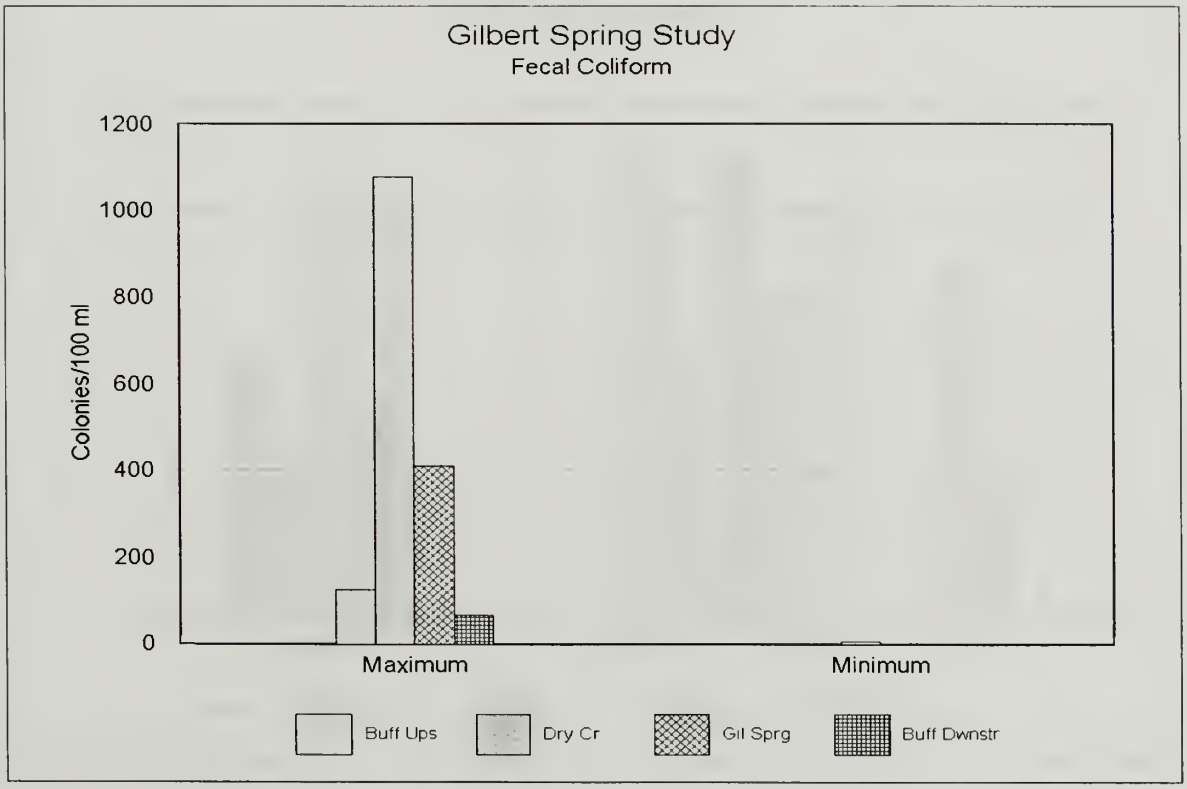
(3) = Samplers lost to vandalism or flooding

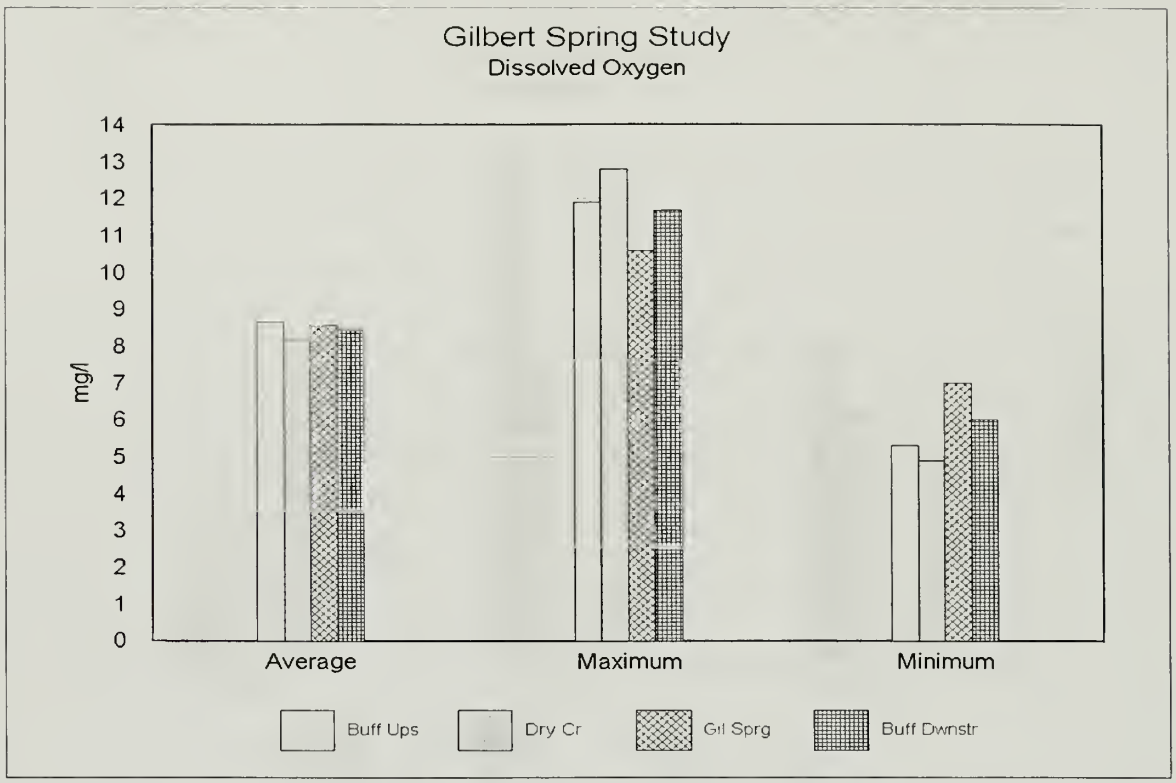
q = Difference in dates and times are according to the Buffalo National River sample collection data sheets.

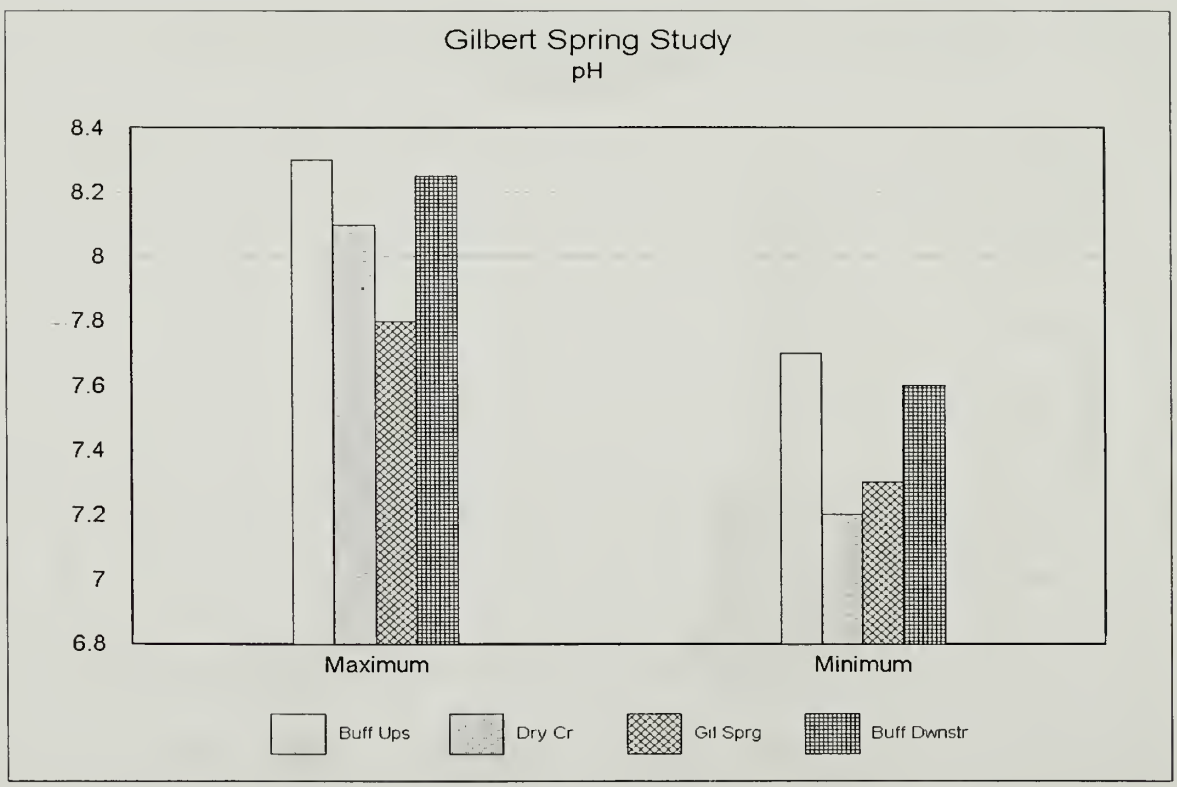
Appendix E
Water Quality Graphics

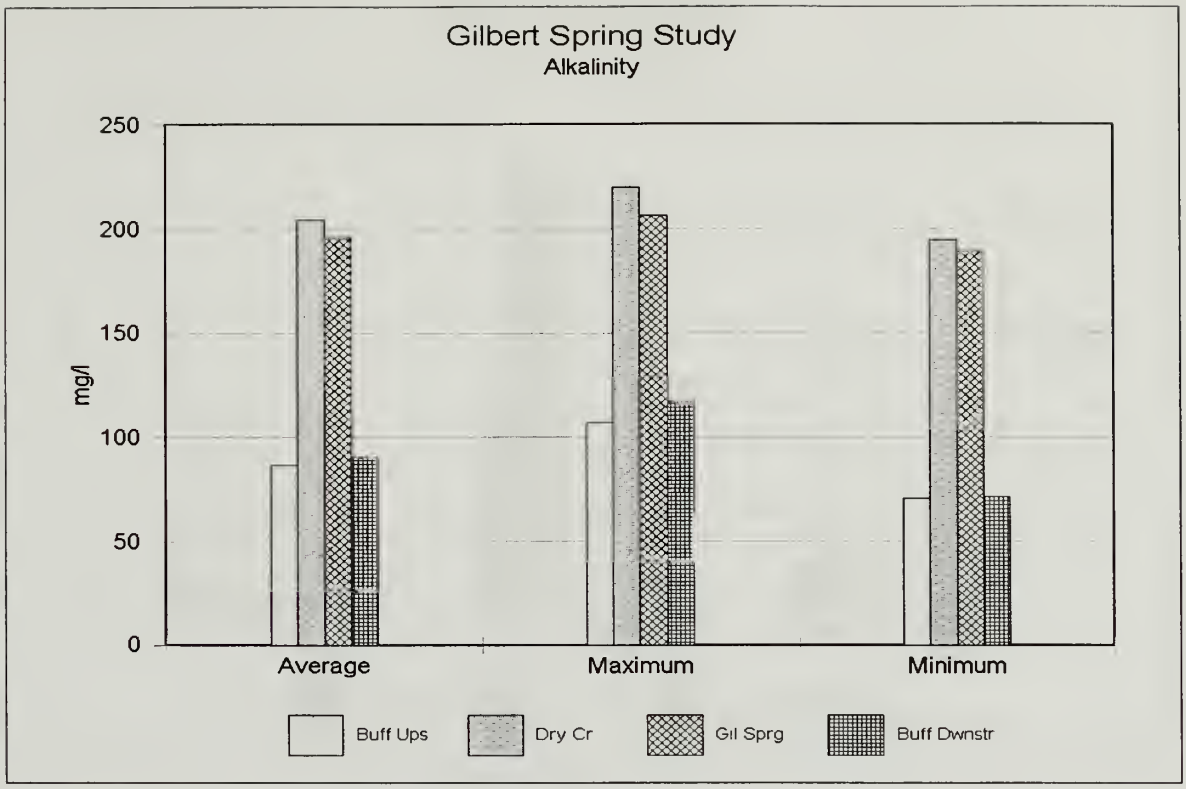
Appendix E1
Storm Event Graphs

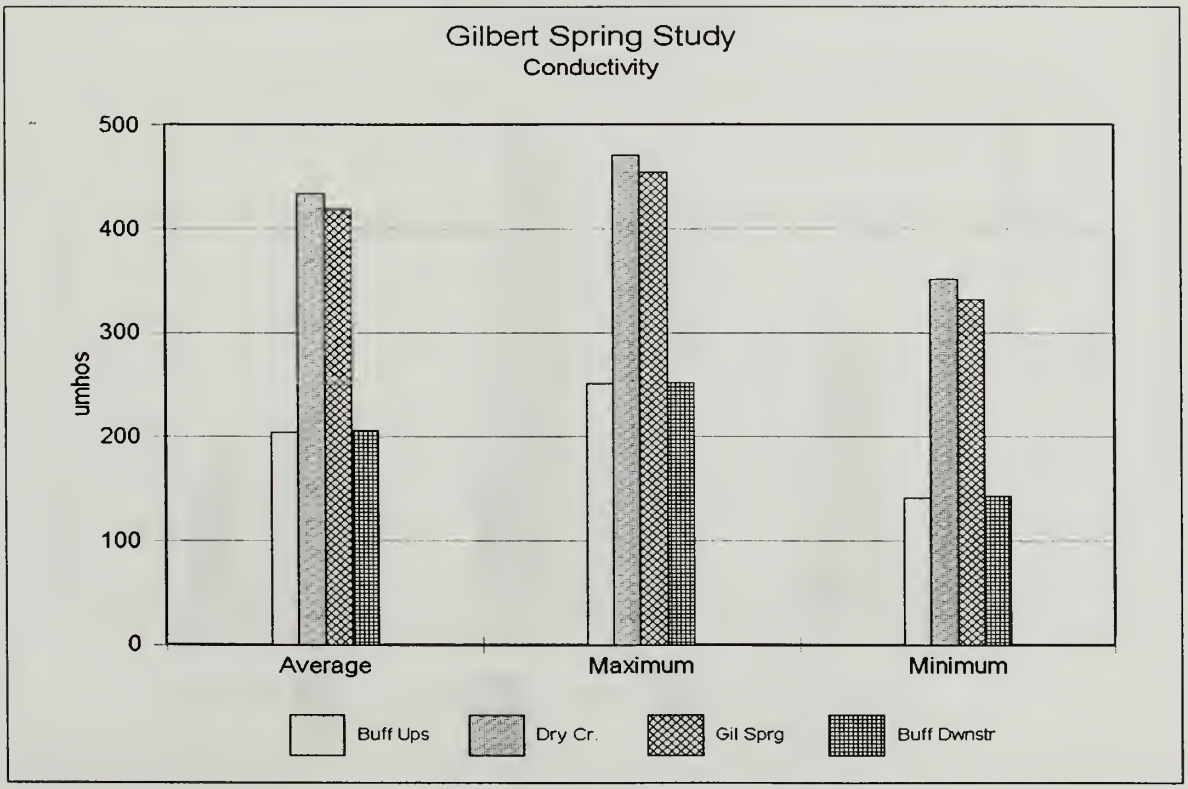

Gilbert Spring Study
Water Temperature
Degrees Celsius
30
25
20
15
10
5
0
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

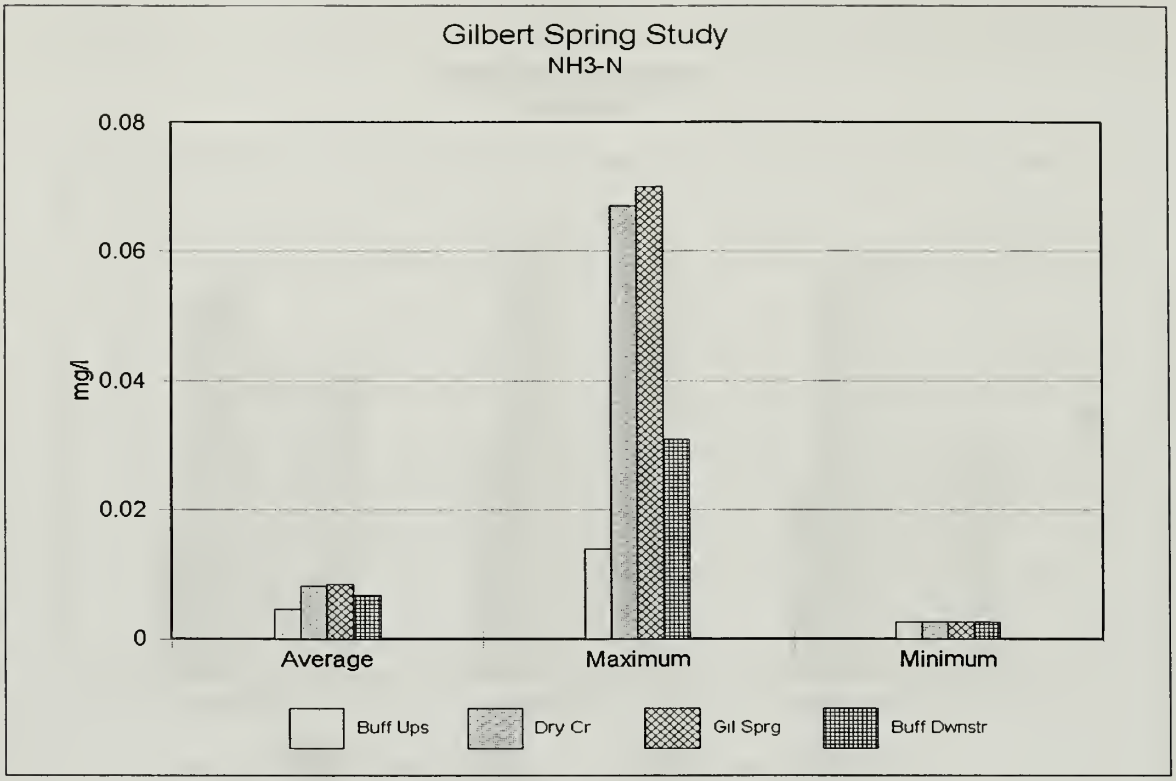

Gilbert Spring Study
Fecal Coliform
Colonies/100 ml
1200
1000
800
600
400
200
0
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

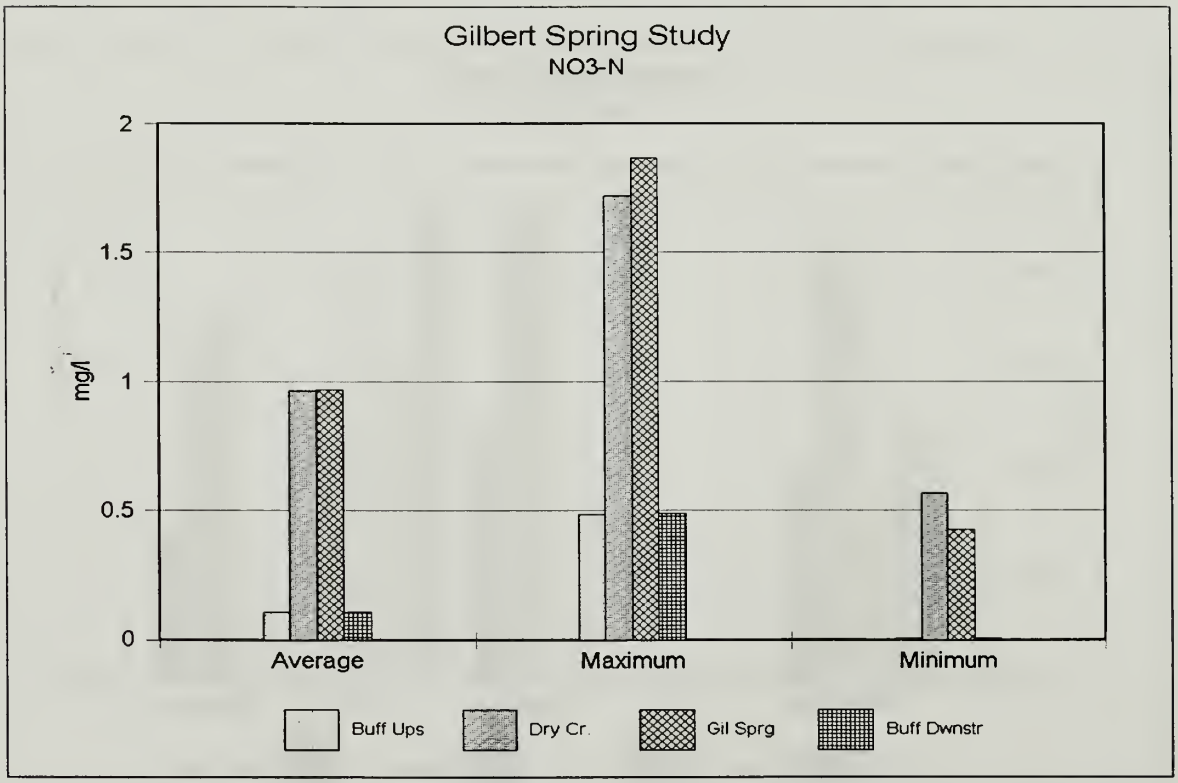

Gilbert Spring Study
Dissolved Oxygen
mg/l
14
13
12
11
10
9
8
7
6
5
4
3
2
1
0
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

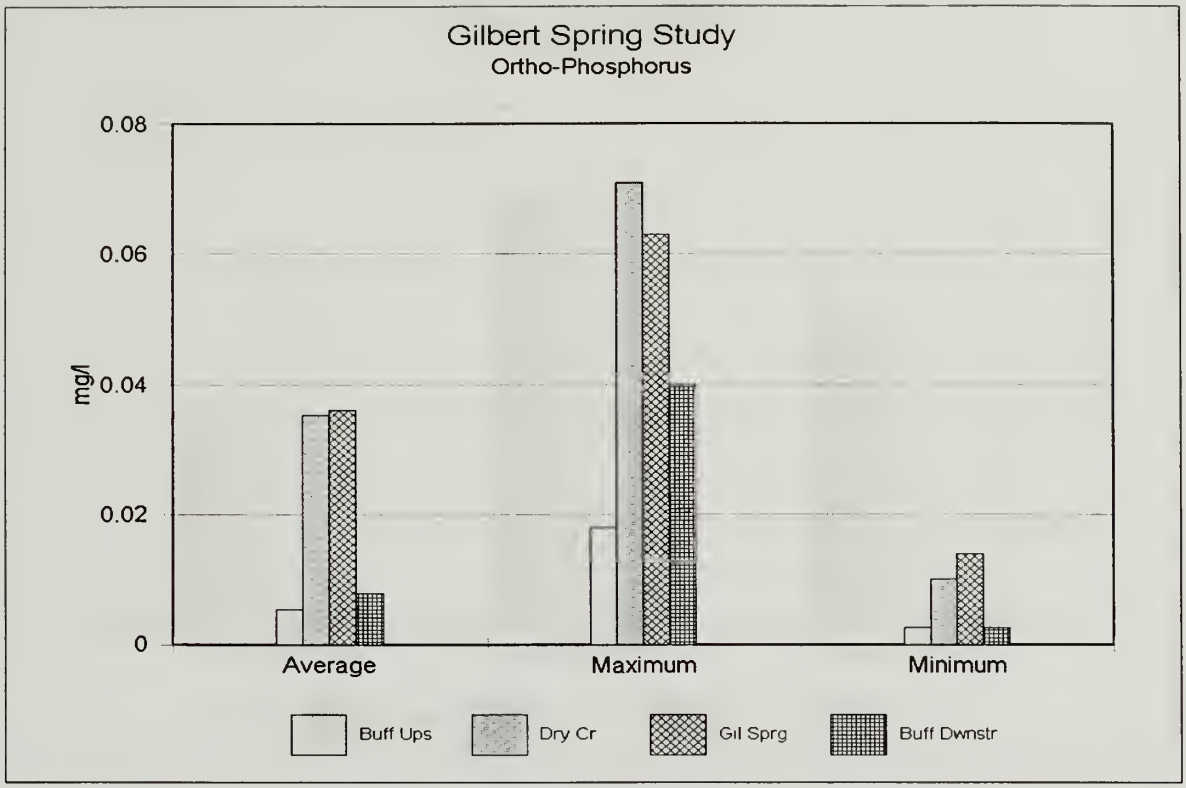

Gilbert Spring Study
pH
8.4
8.2
8
7.8
7.6
7.4
7.2
7
6.8
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

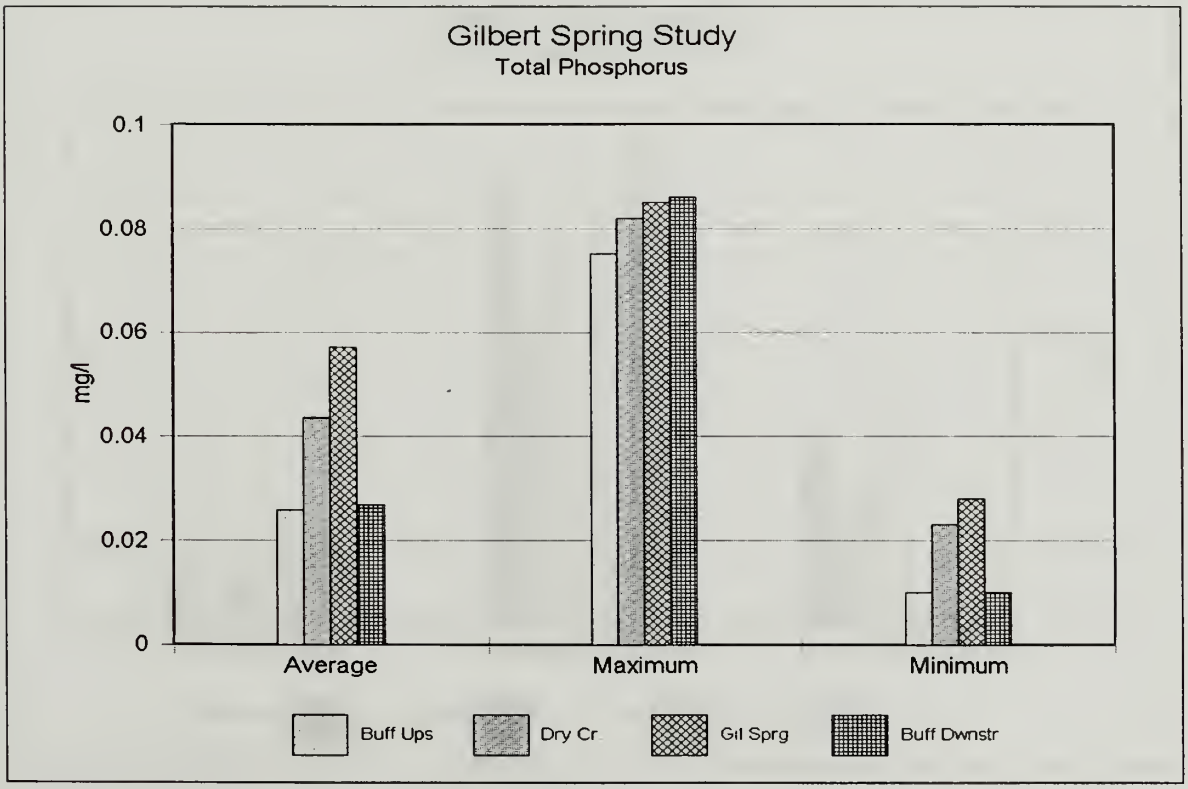

Gilbert Spring Study
Alkalinity
250
200
150
100
50
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

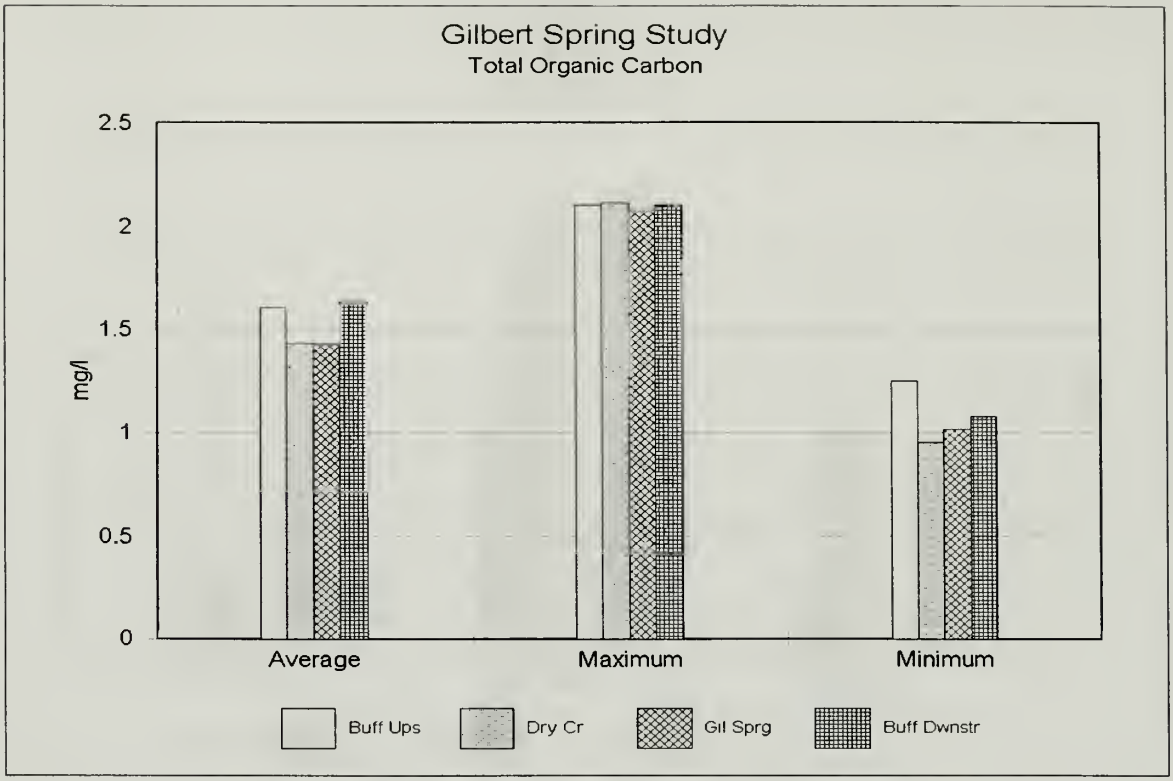

Gilbert Spring Study
Conductivity
500
400
300
200
100
0
umhos
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

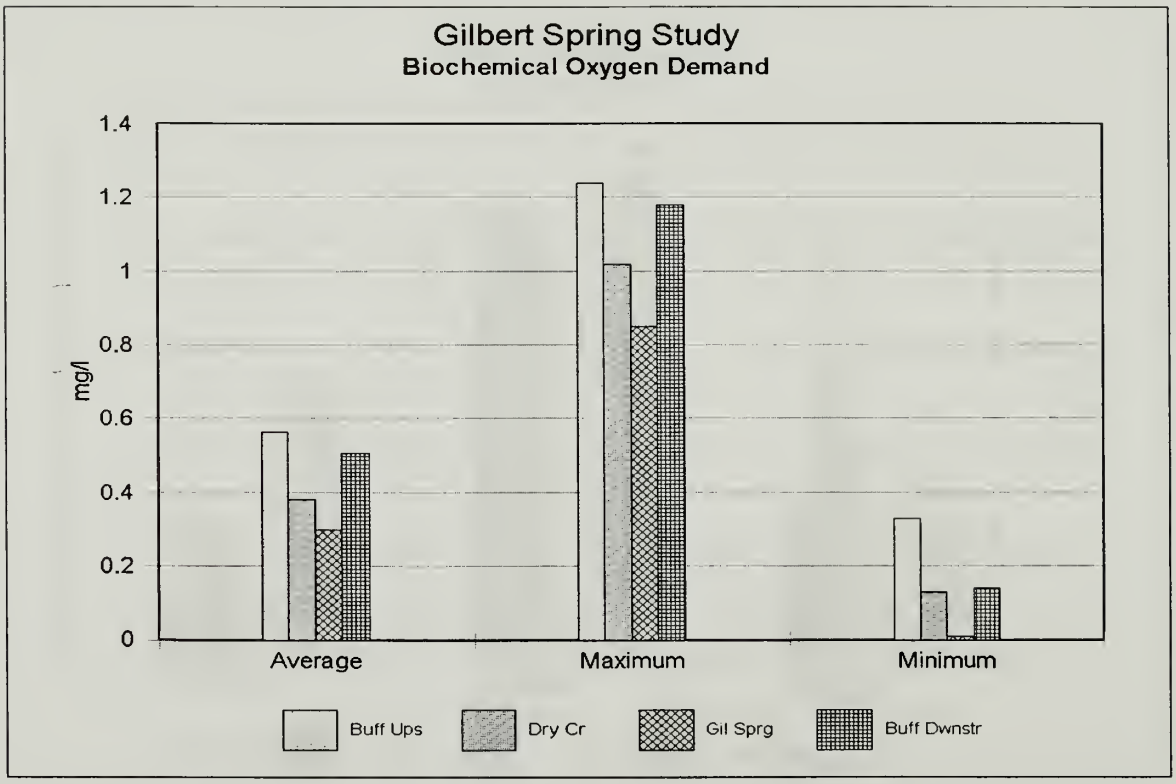

Gilbert Spring Study
NH3-N
0.08
0.06
0.04
0.02
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

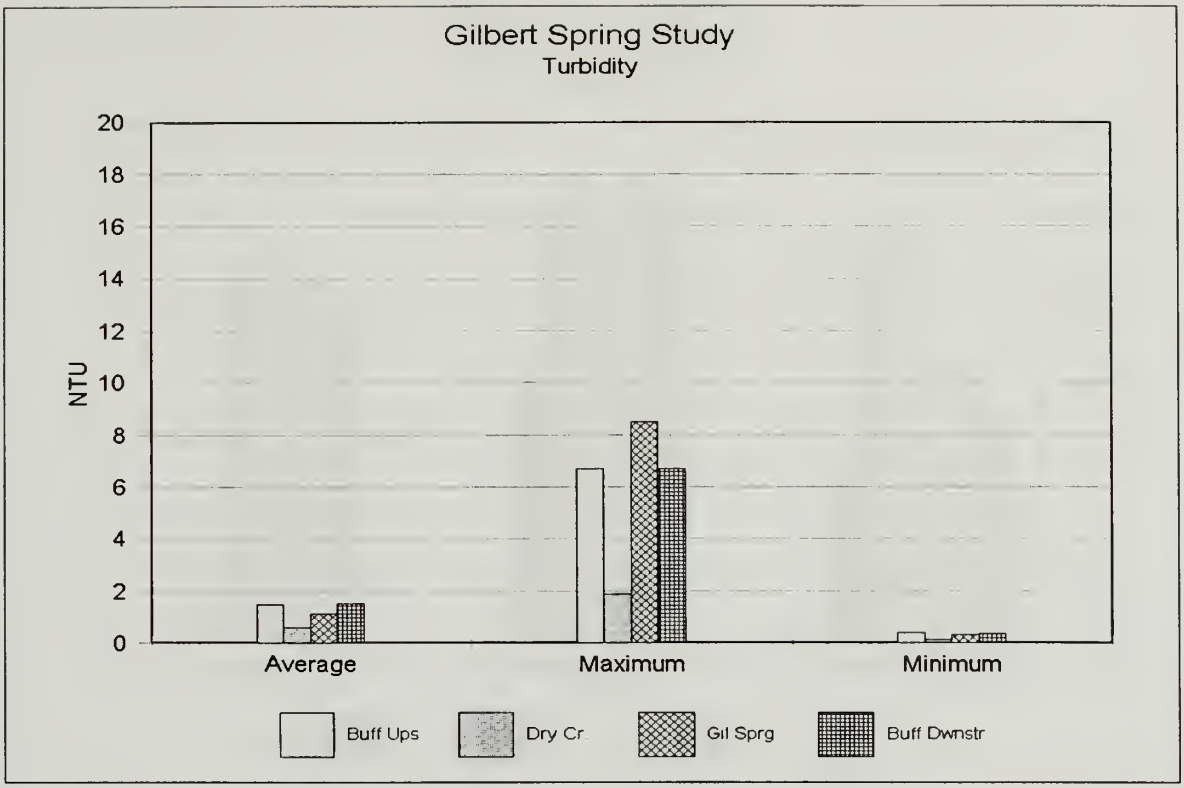

Gilbert Spring Study
NO3-N
2
1.5
1
0.5
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

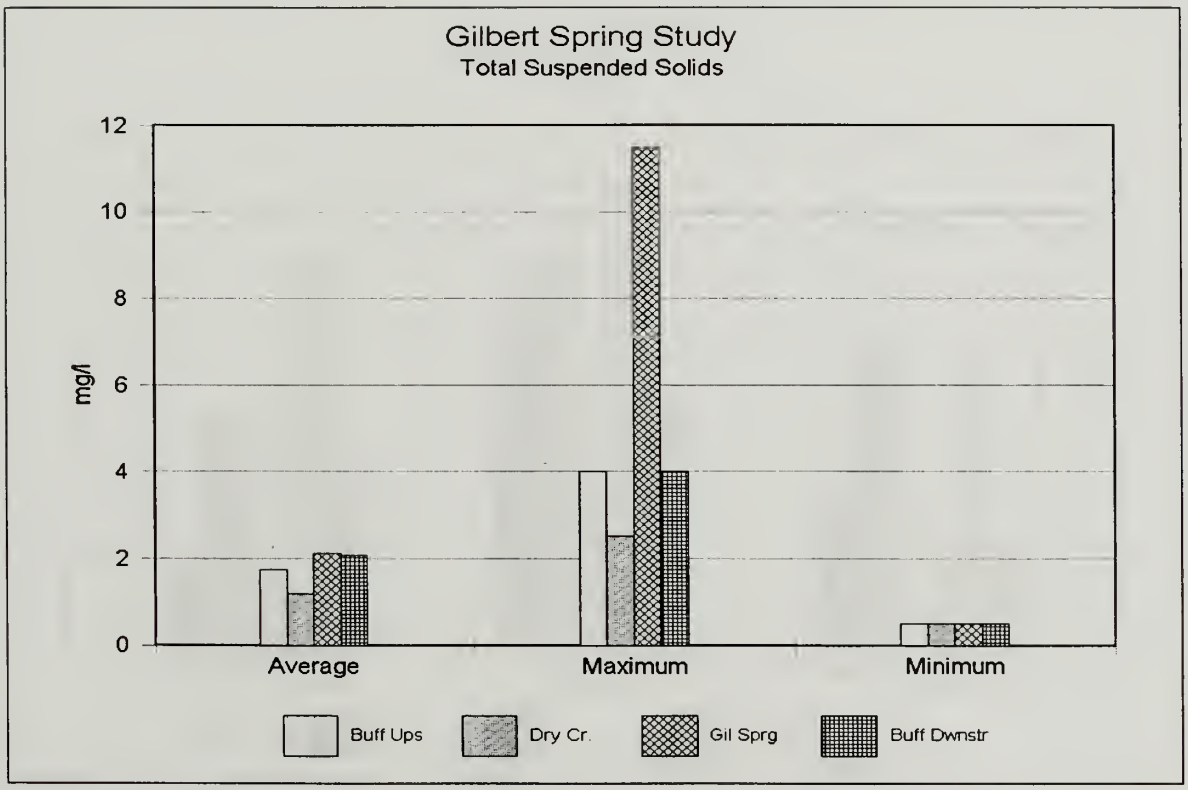

Gilbert Spring Study
Ortho-Phosphorus
0.08
0.06
0.04
0.02
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

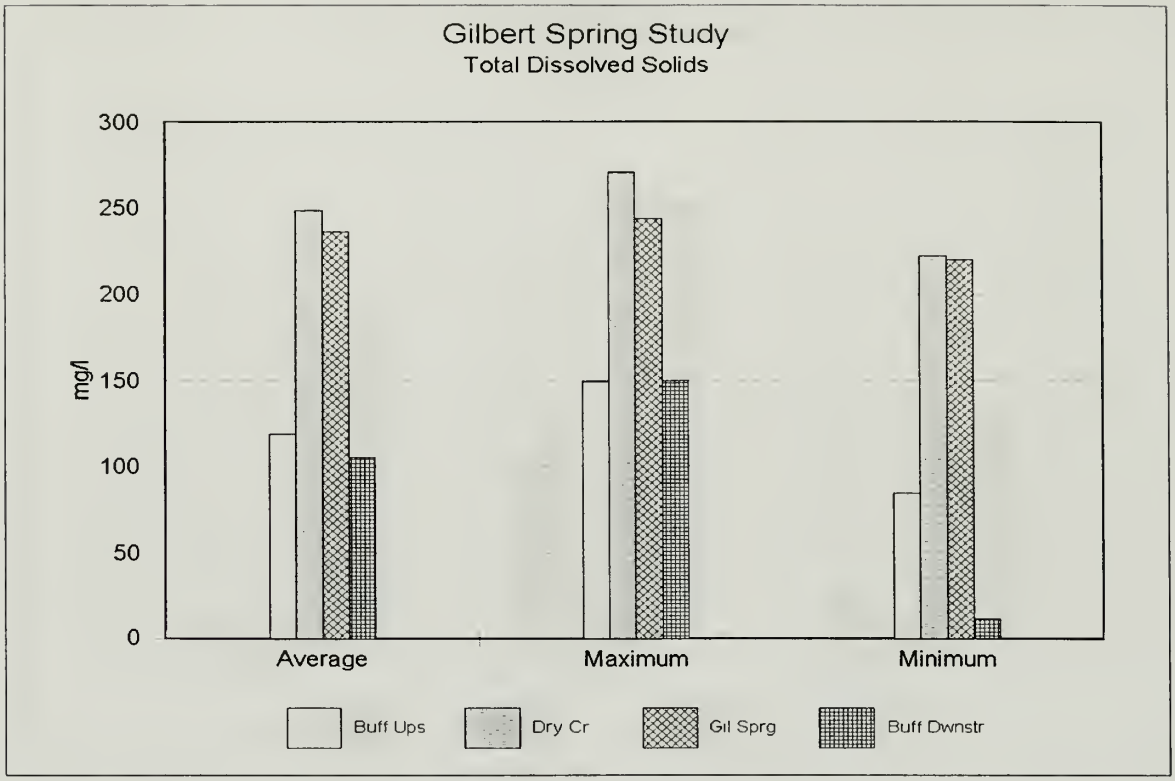

Gilbert Spring Study
Total Phosphorus
0.1
0.08
0.06
0.04
0.02
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

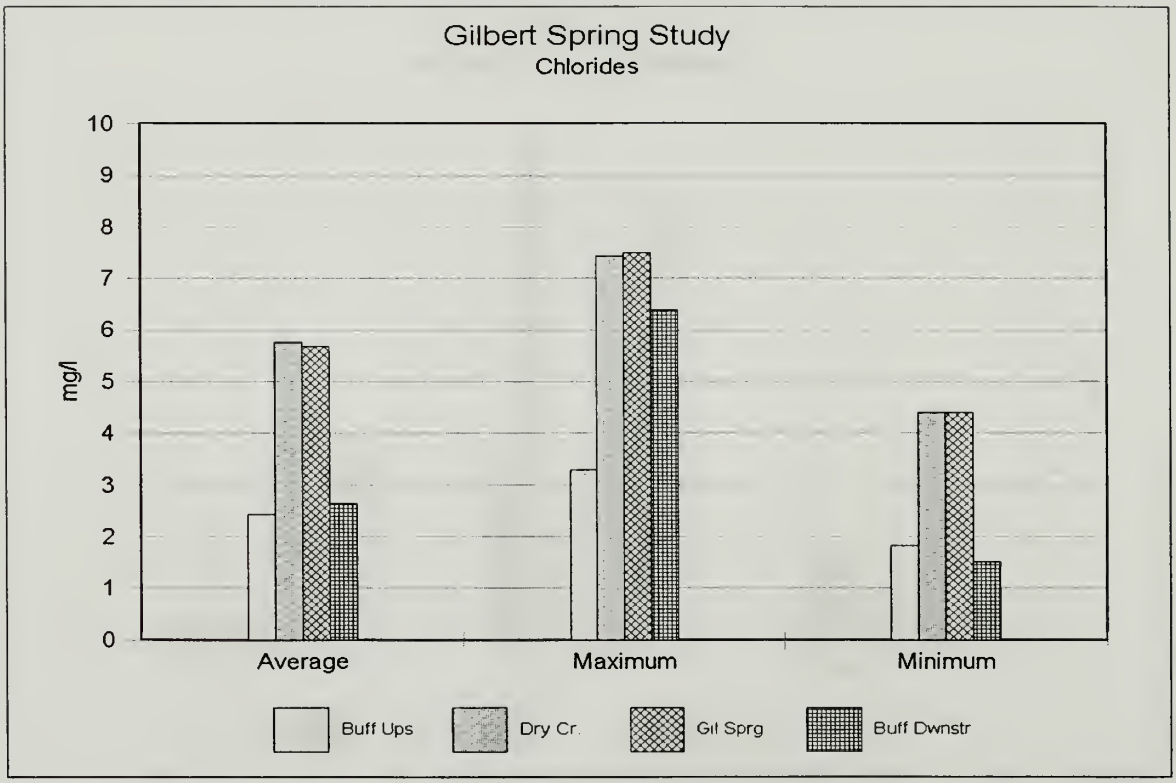

Gilbert Spring Study
Total Organic Carbon
2.5
2
1.5
1
0.5
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

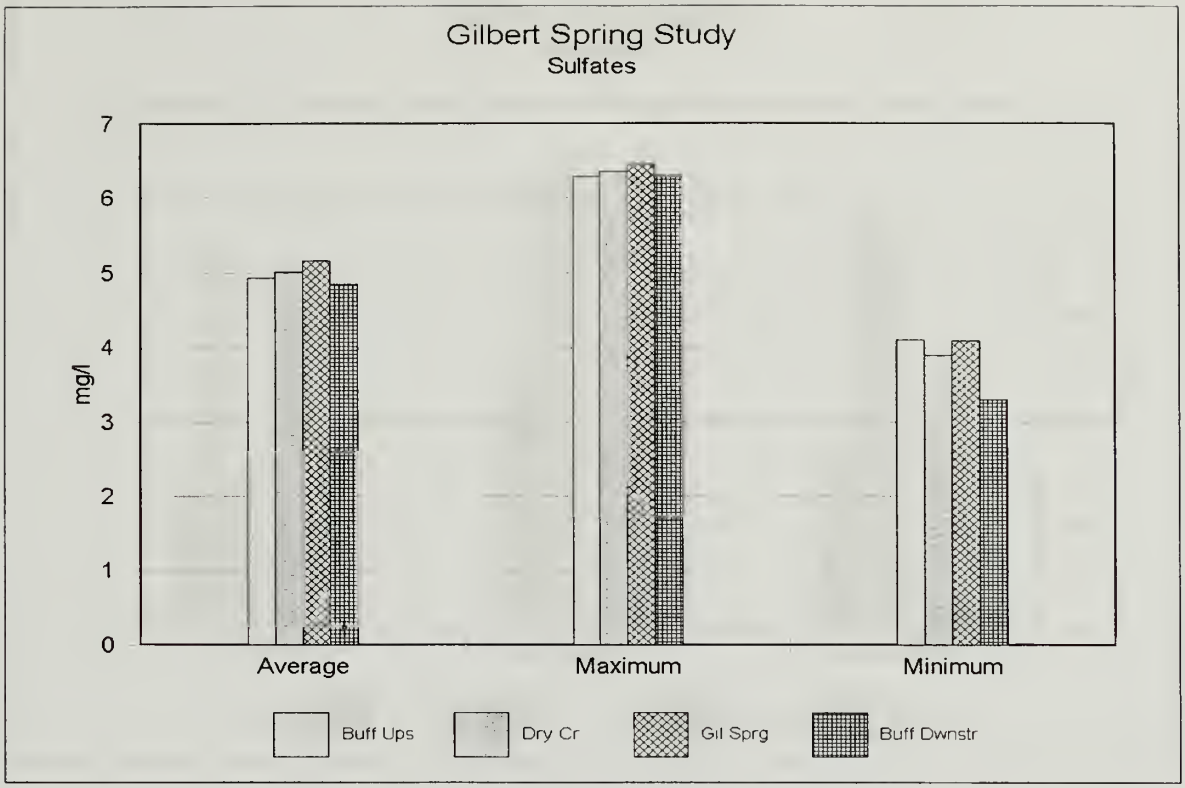

Gilbert Spring Study
Biochemical Oxygen Demand
1.4
1.2
1
0.8
0.6
0.4
0.2
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

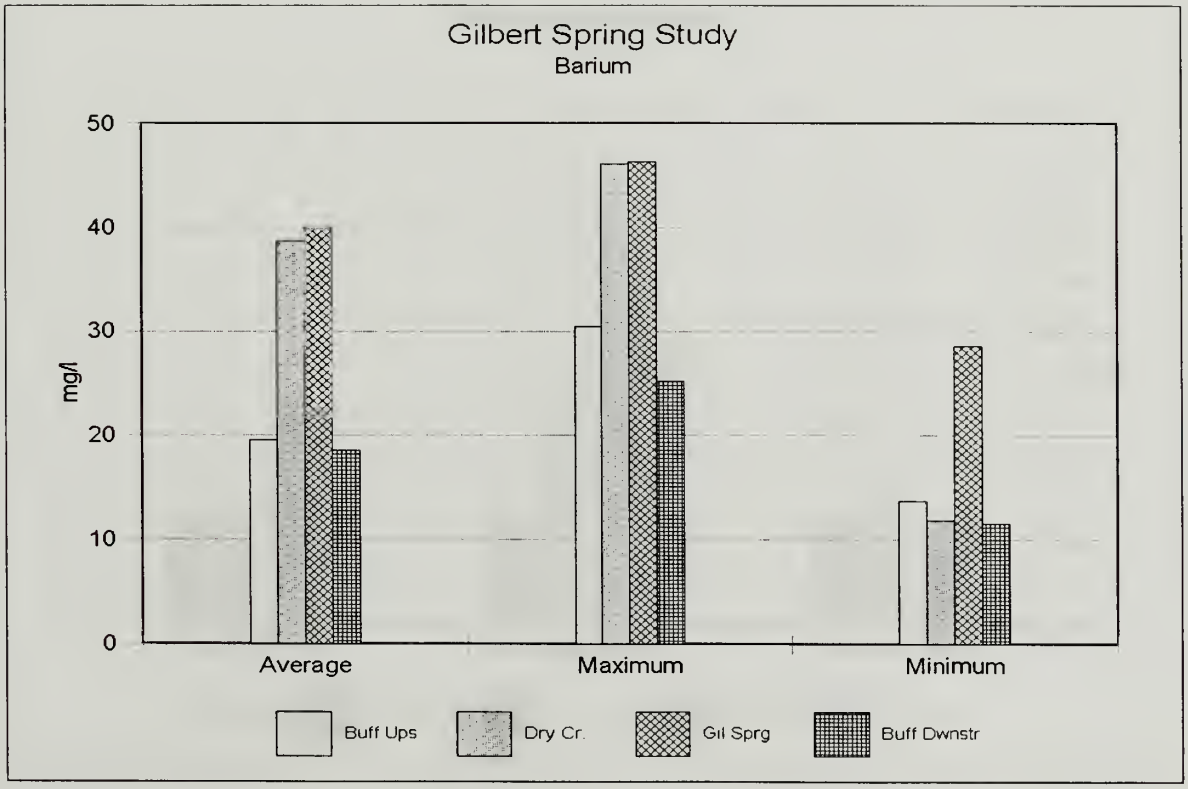

Gilbert Spring Study
Turbidity
NTU
20
18
16
14
12
10
8
6
4
2
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

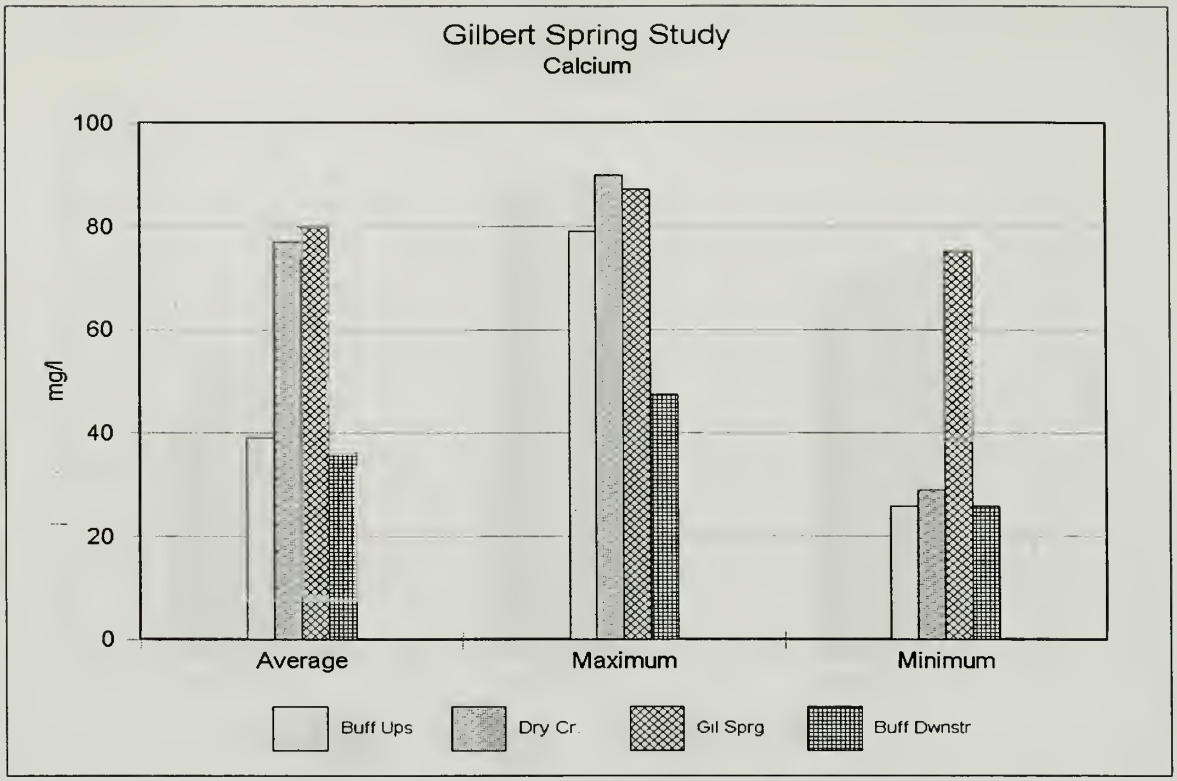

Gilbert Spring Study
Total Suspended Solids
mg/l
12
10
8
6
4
2
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

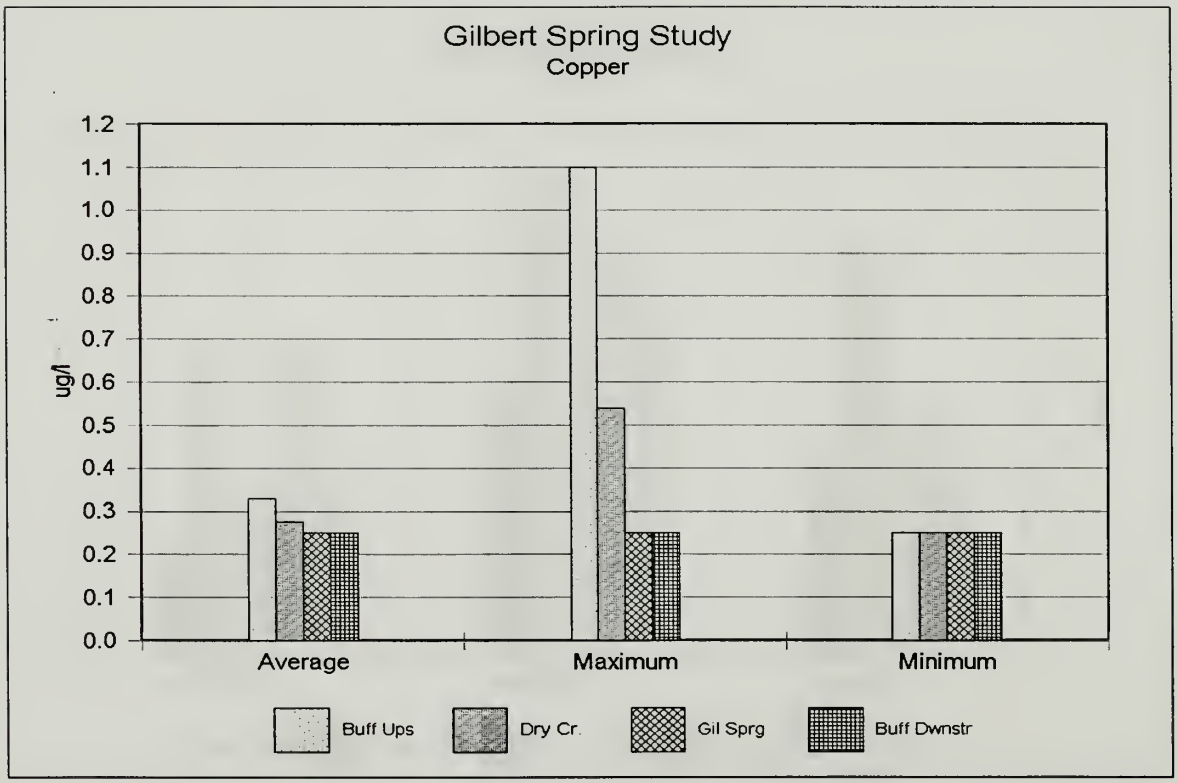

Gilbert Spring Study
Total Dissolved Solids
300
250
200
150
100
50
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

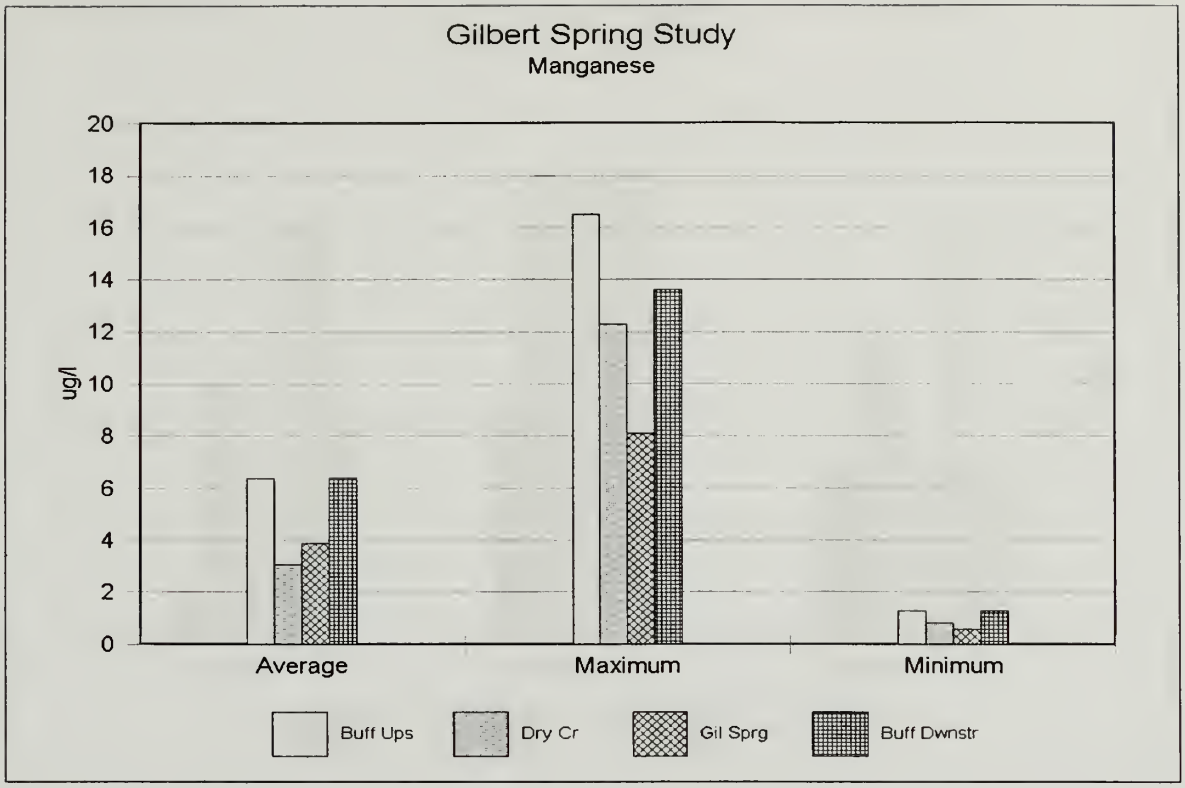

Gilbert Spring Study
Chlorides
10
9
8
7
6
5
4
3
2
1
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

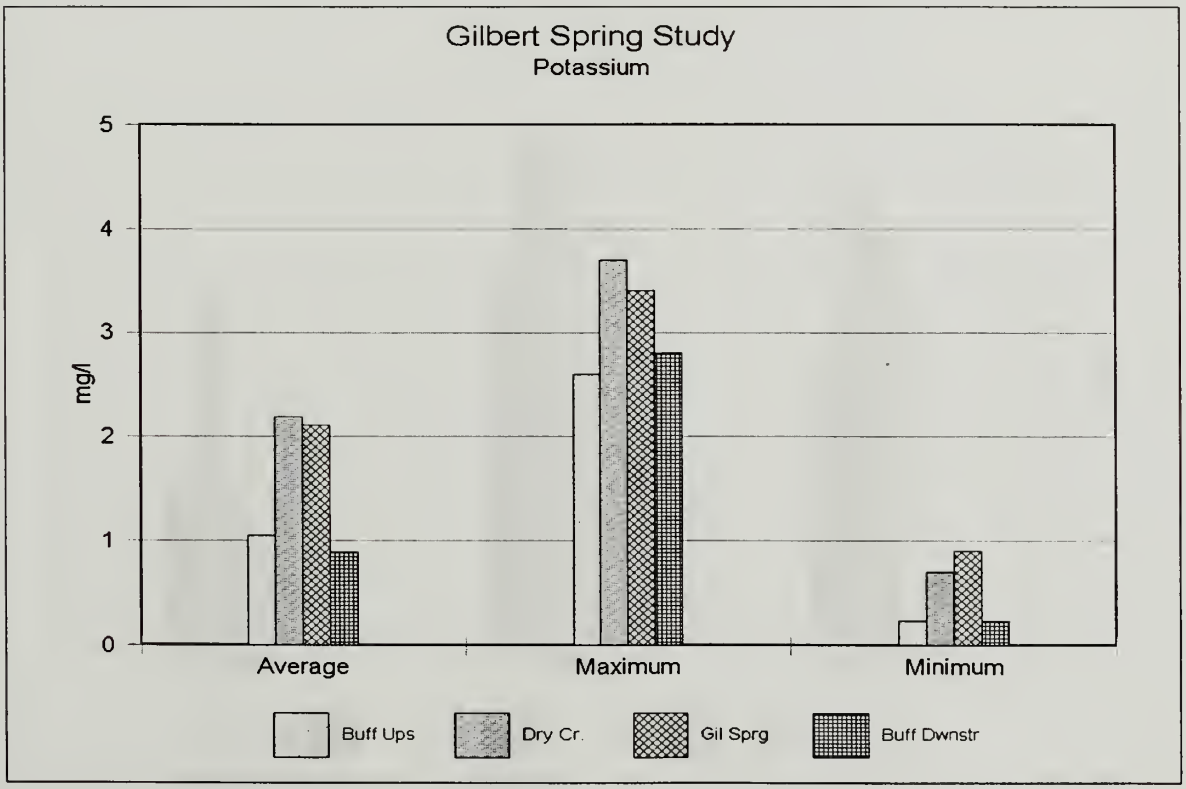

Gilbert Spring Study
Sulfates
mg/l
7
6
5
4
3
2
1
0
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

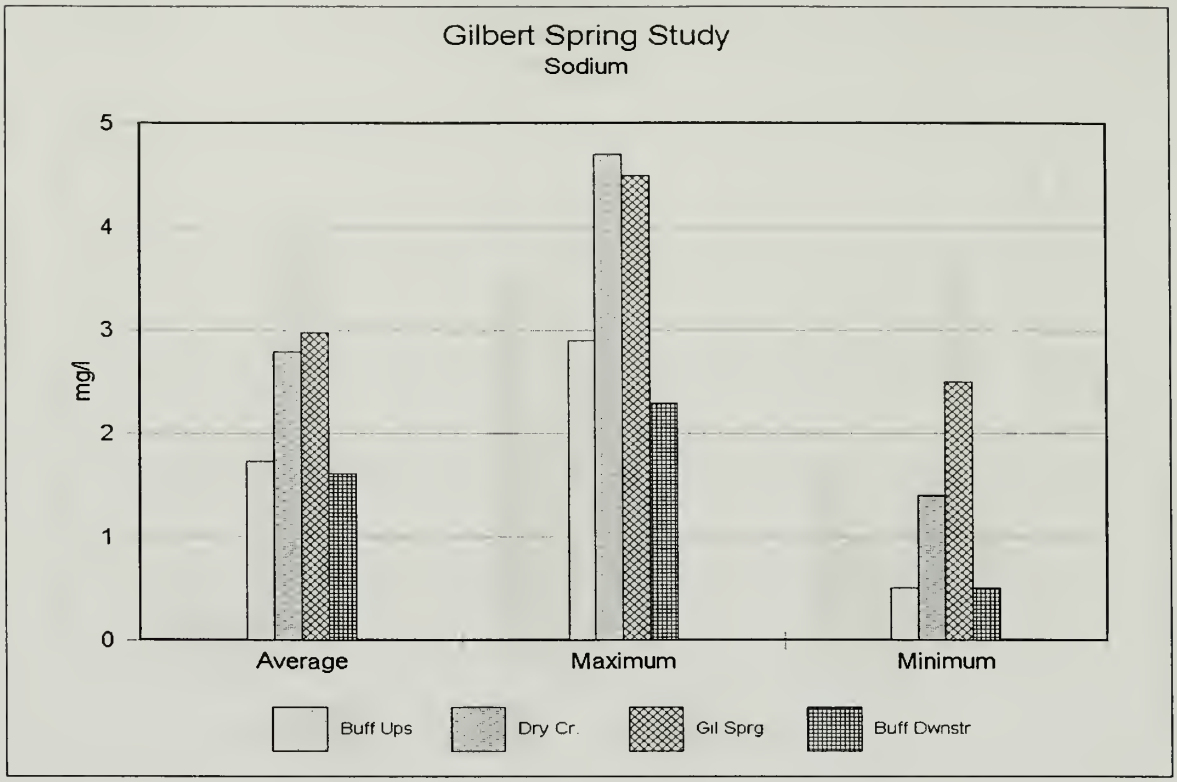

Gilbert Spring Study
Barium
mg/l
50
40
30
20
10
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

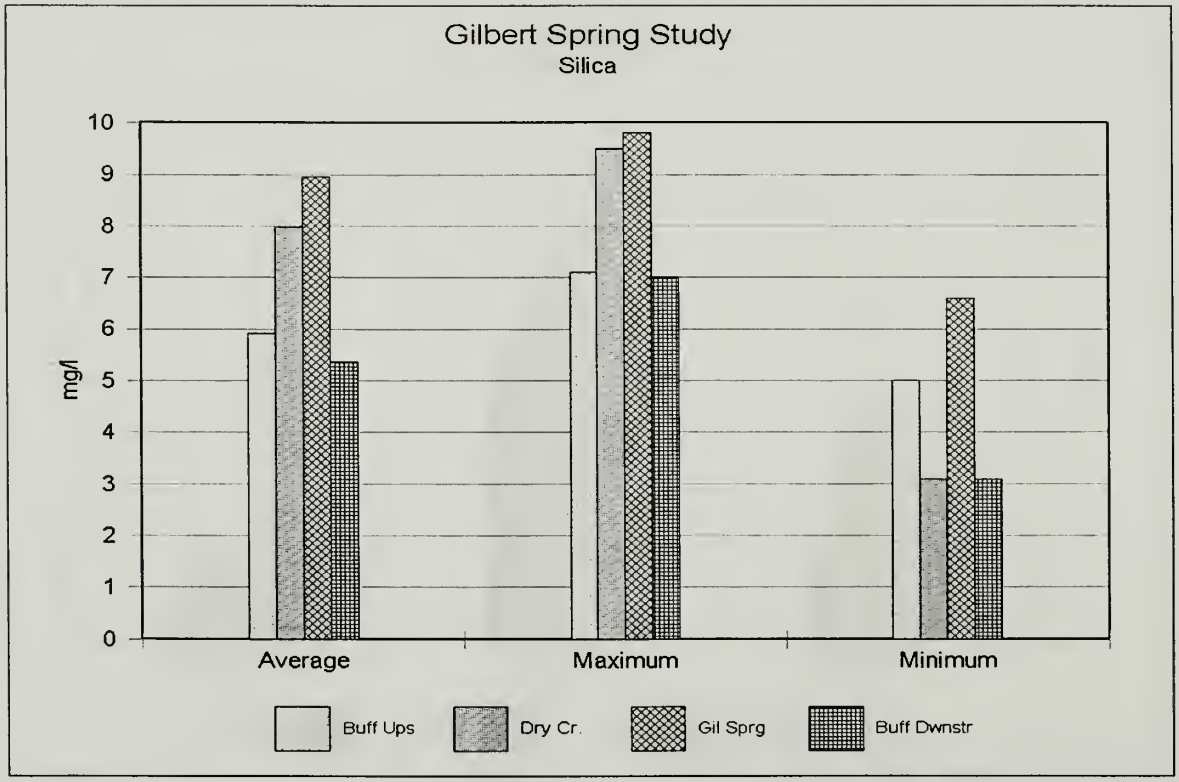

Gilbert Spring Study
Calcium
100
80
60
40
20
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

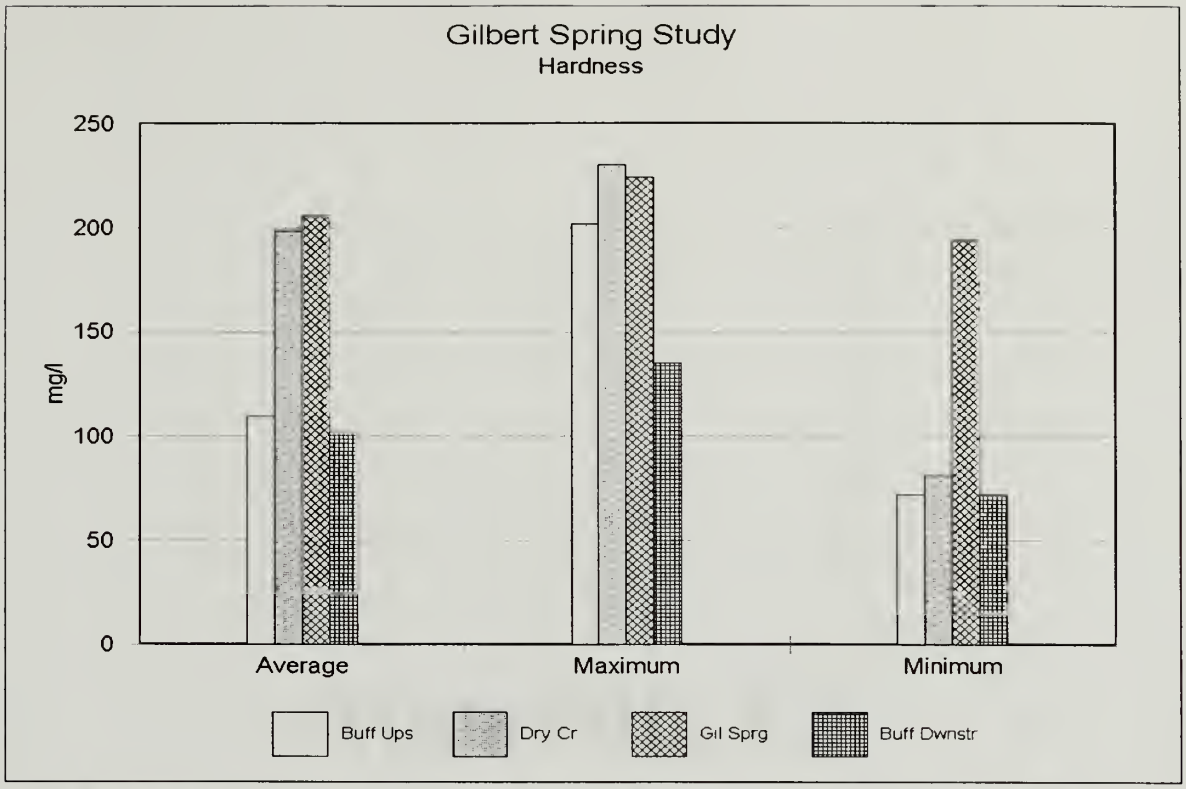

Gilbert Spring Study
Copper
1.2
1.1
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
ug/l
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr


Gilbert Spring Study
Manganese
ug/l
20
18
16
14
12
10
8
6
4
2
0
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

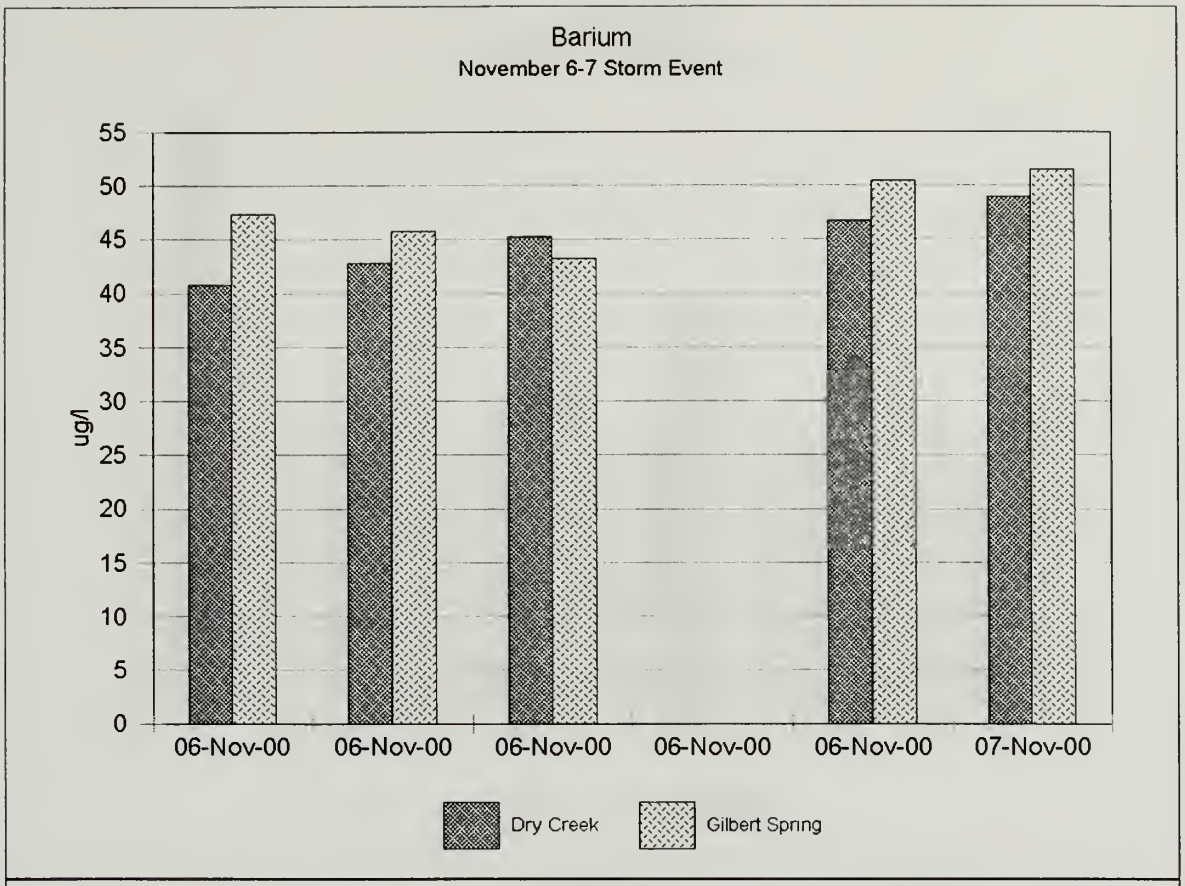

Gilbert Spring Study
Potassium
mg/l
5
4
3
2
1
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

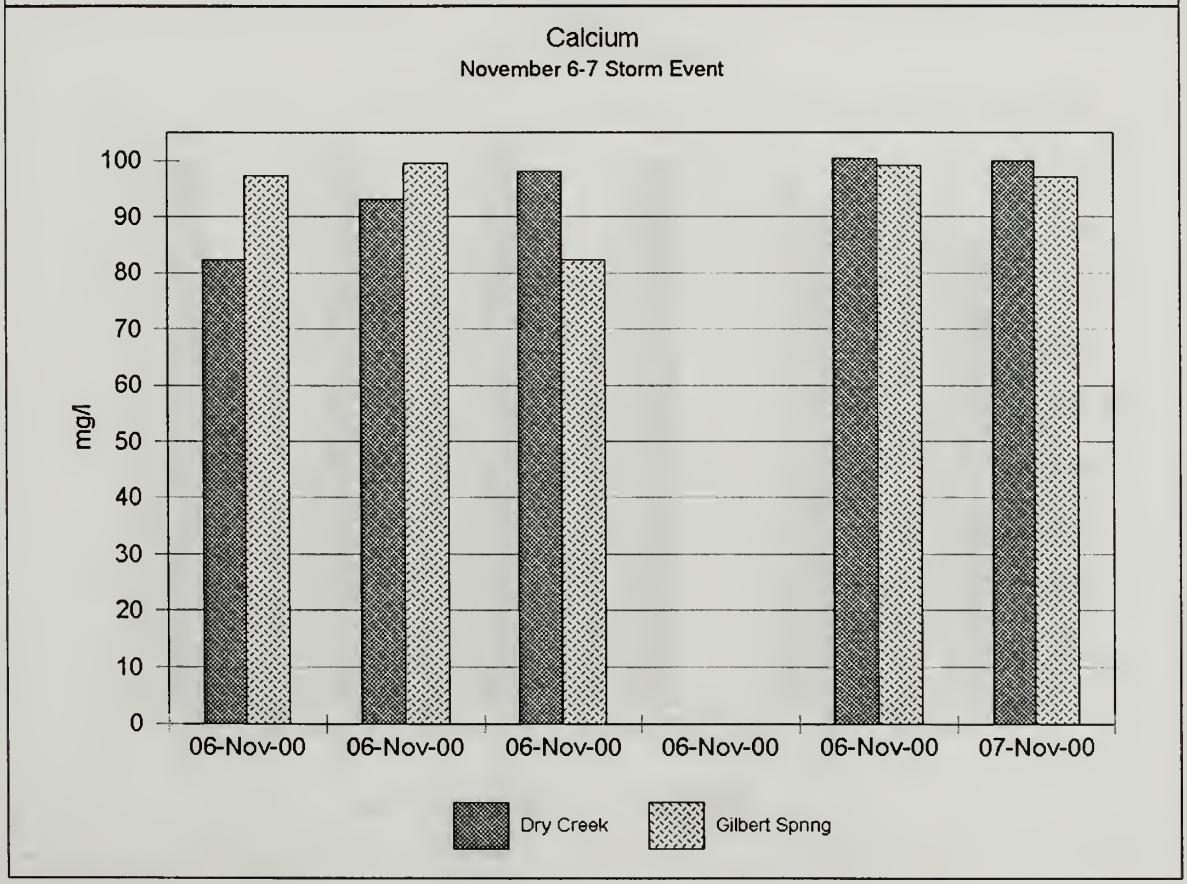

Gilbert Spring Study
Sodium
mg/l
5
4
3
2
1
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

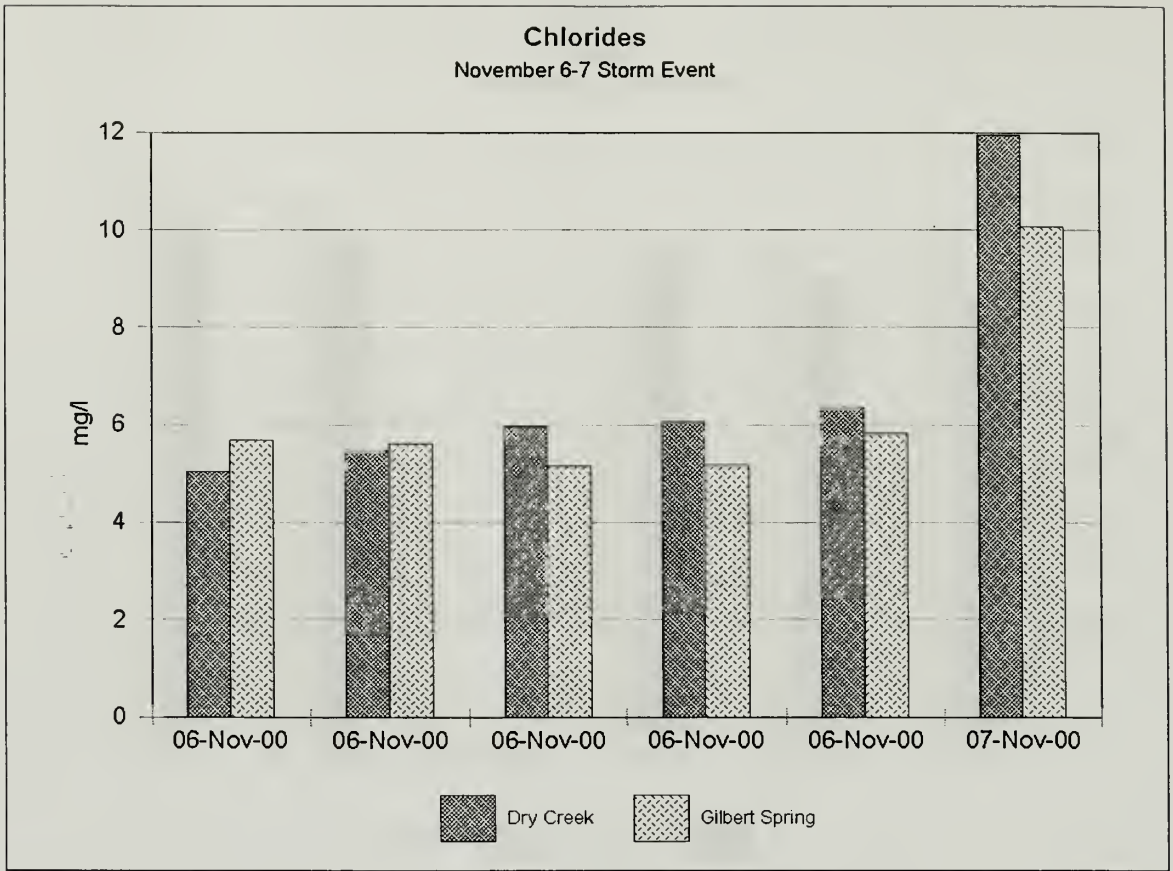

Gilbert Spring Study
Silica
mg/l
10
9
8
7
6
5
4
3
2
1
0
Average
Maximum
Minimum
Buff Ups
Dry Cr.
Gil Sprg
Buff Dwnstr

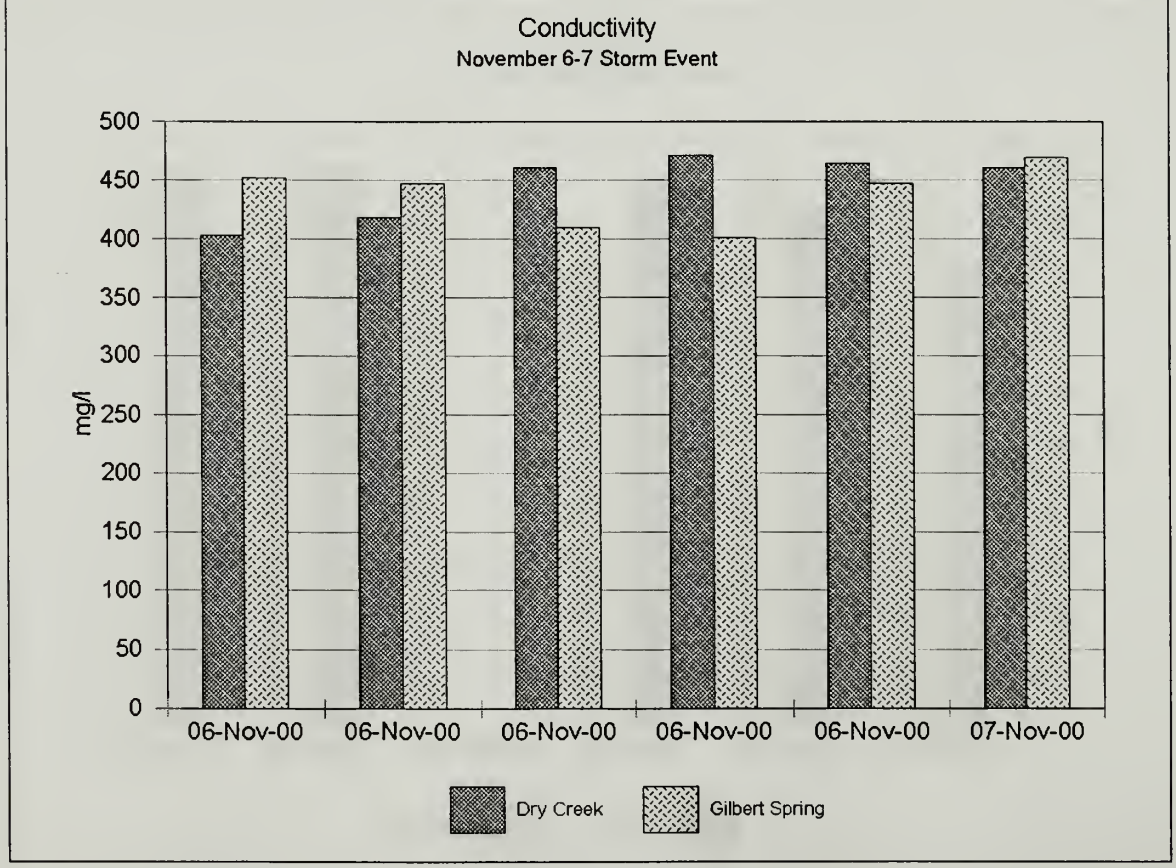

Gilbert Spring Study
Hardness
250
200
150
100
50
0
mg/l
Average
Maximum
Minimum
Buff Ups
Dry Cr
Gil Sprg
Buff Dwnstr

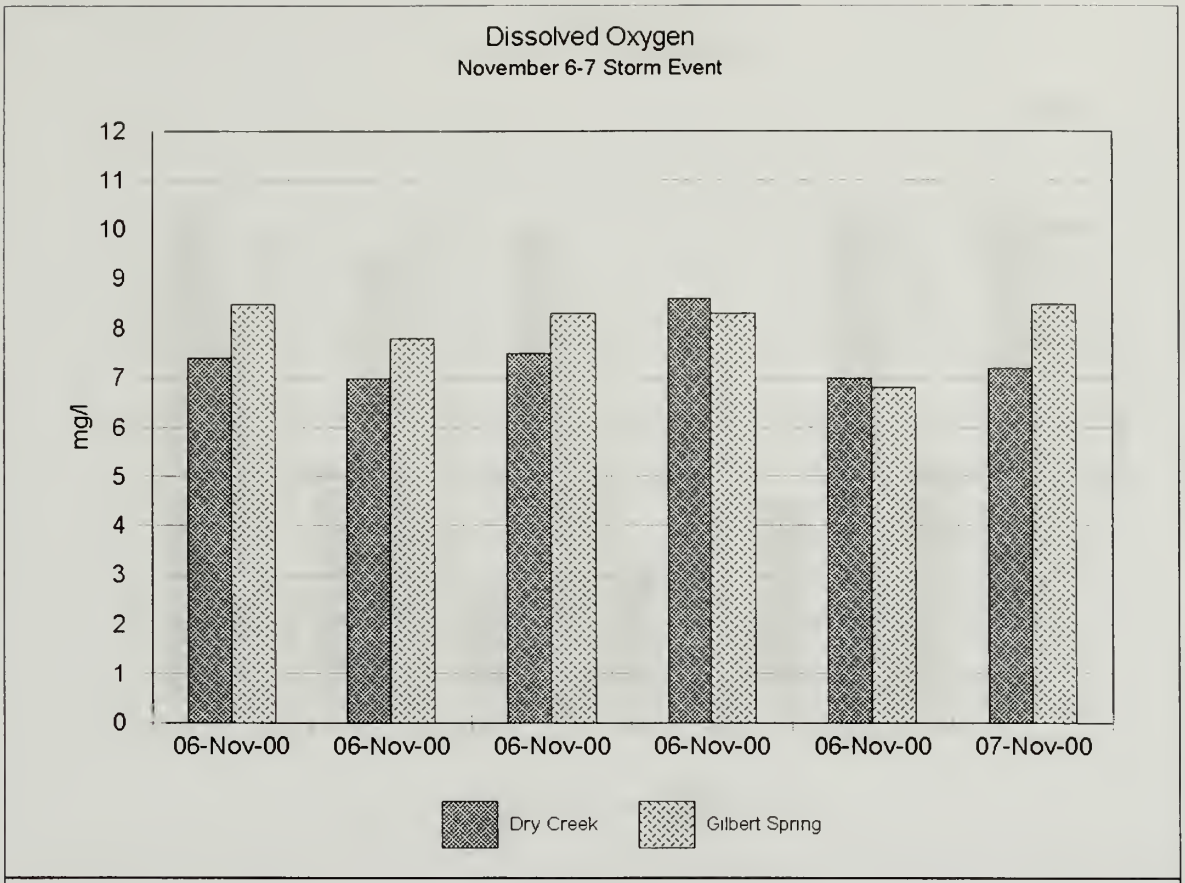
Appendix E2
Routine Sampling Graphs

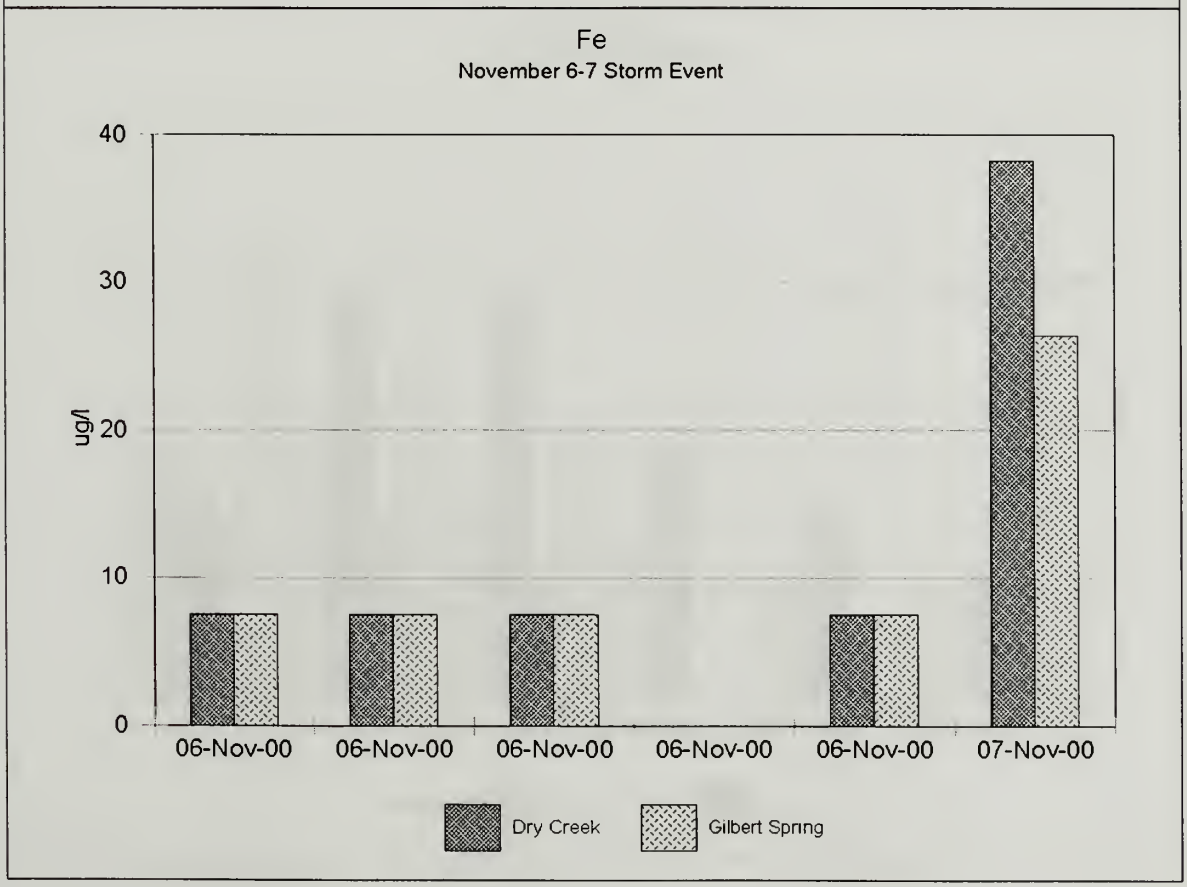

Barium
November 6-7 Storm Event
ug/l
55
50
45
40
35
30
25
20
15
10
5
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

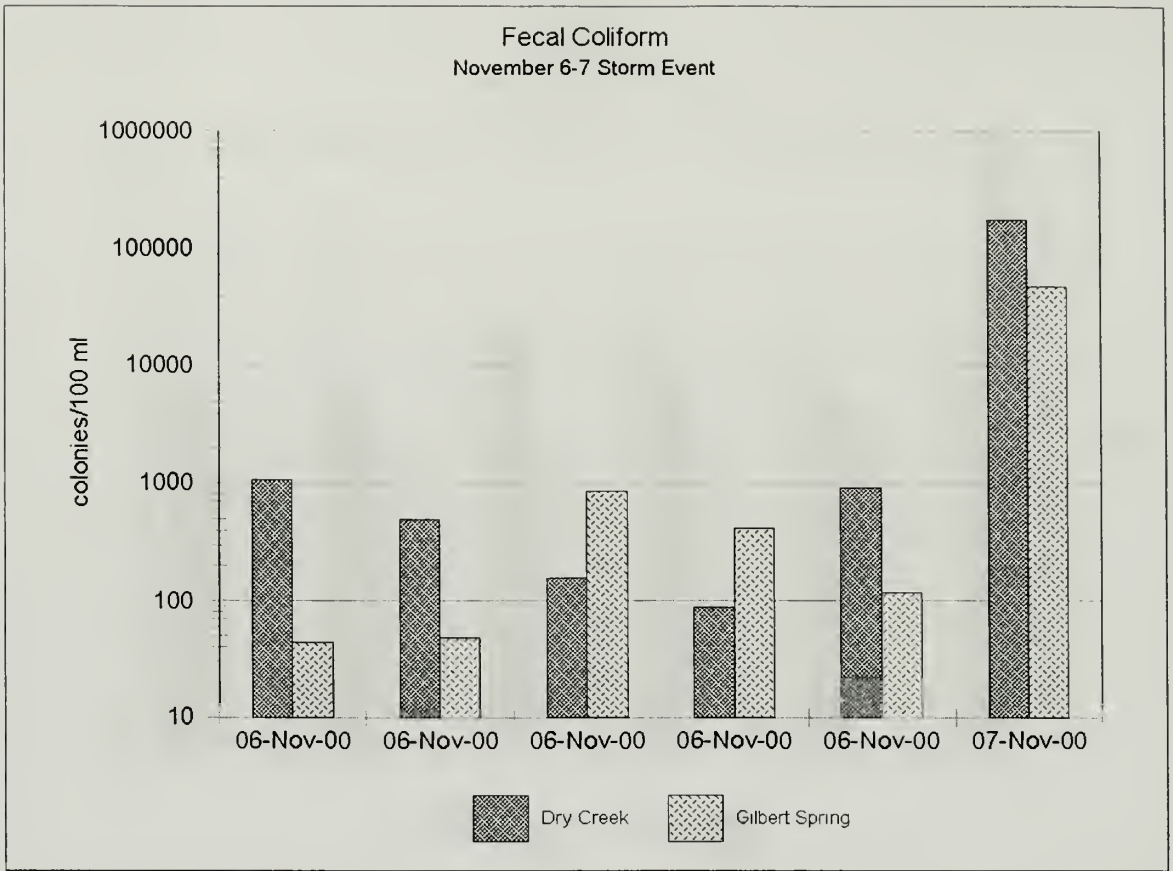

Calcium
November 6-7 Storm Event
mg/l
100
90
80
70
60
50
40
30
20
10
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

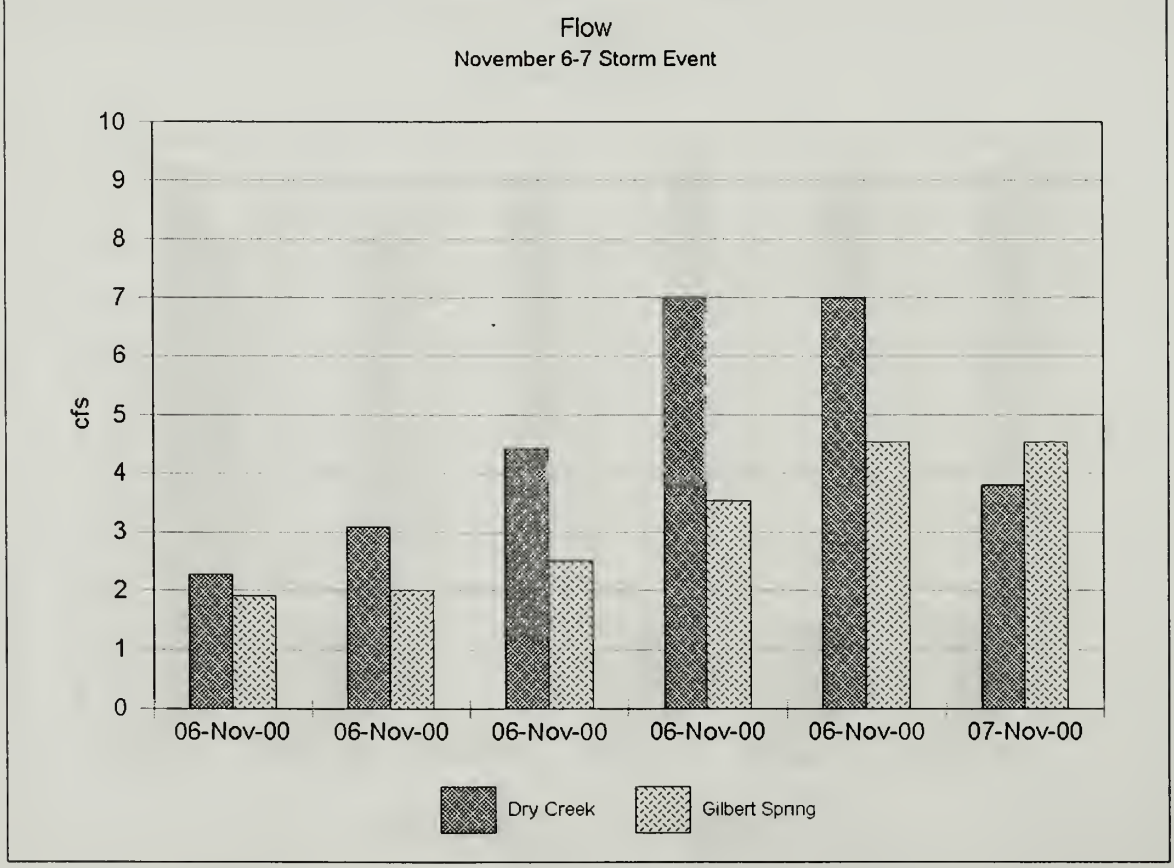

Chlorides
November 6-7 Storm Event
mg/l
12
10
8
6
4
2
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Conductivity
November 6-7 Storm Event
mg/l
500
450
400
350
300
250
200
150
100
50
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Dissolved Oxygen
November 6-7 Storm Event
mg/l
12
11
10
9
8
7
6
5
4
3
2
1
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

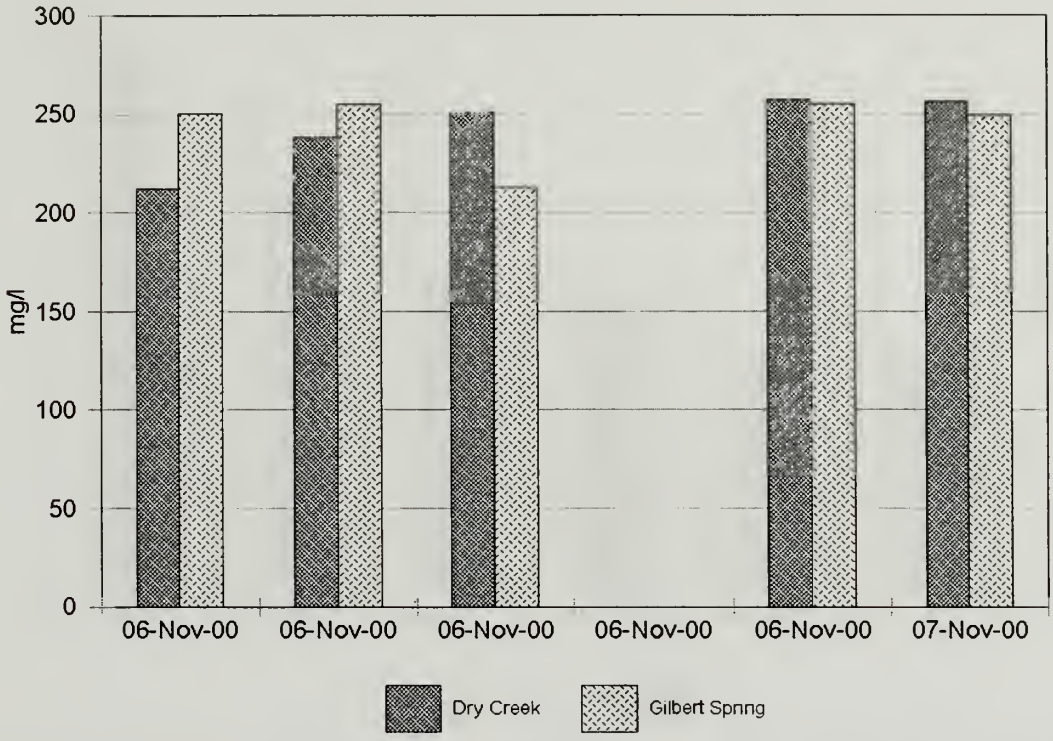

Fe
November 6-7 Storm Event
ug/l
40
30
20
10
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Fecal Coliform
November 6-7 Storm Event
colonies/100 ml
1000000
100000
10000
1000
100
10
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Flow
November 6-7 Storm Event
cfs
10
9
8
7
6
5
4
3
2
1
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

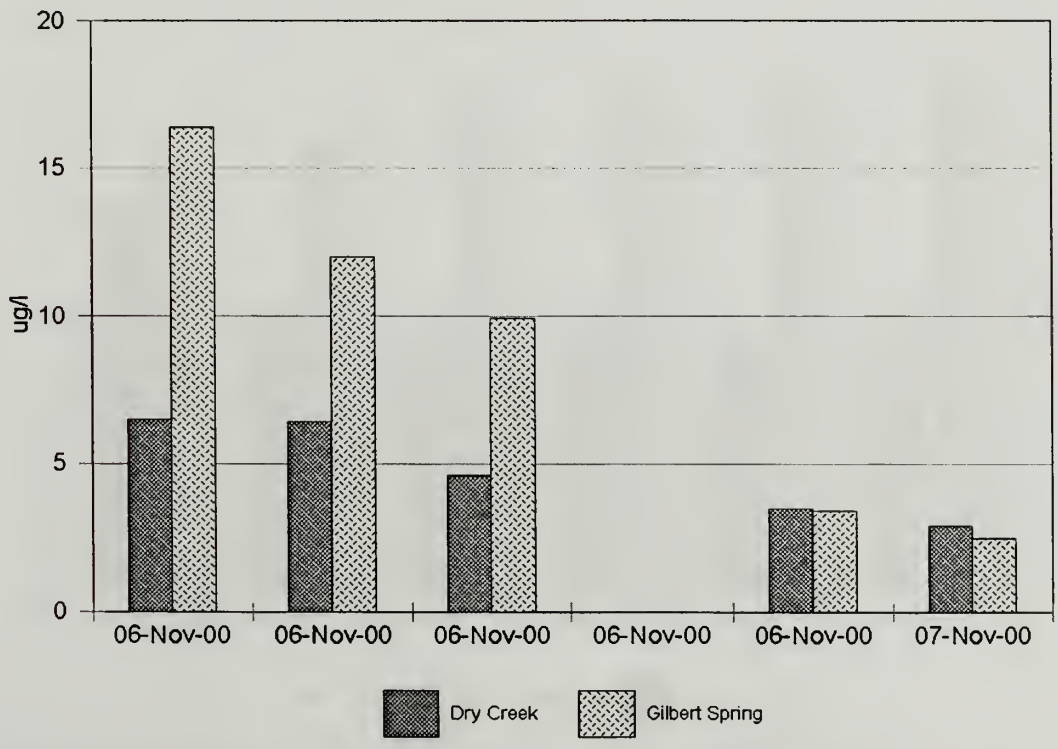
Hardness

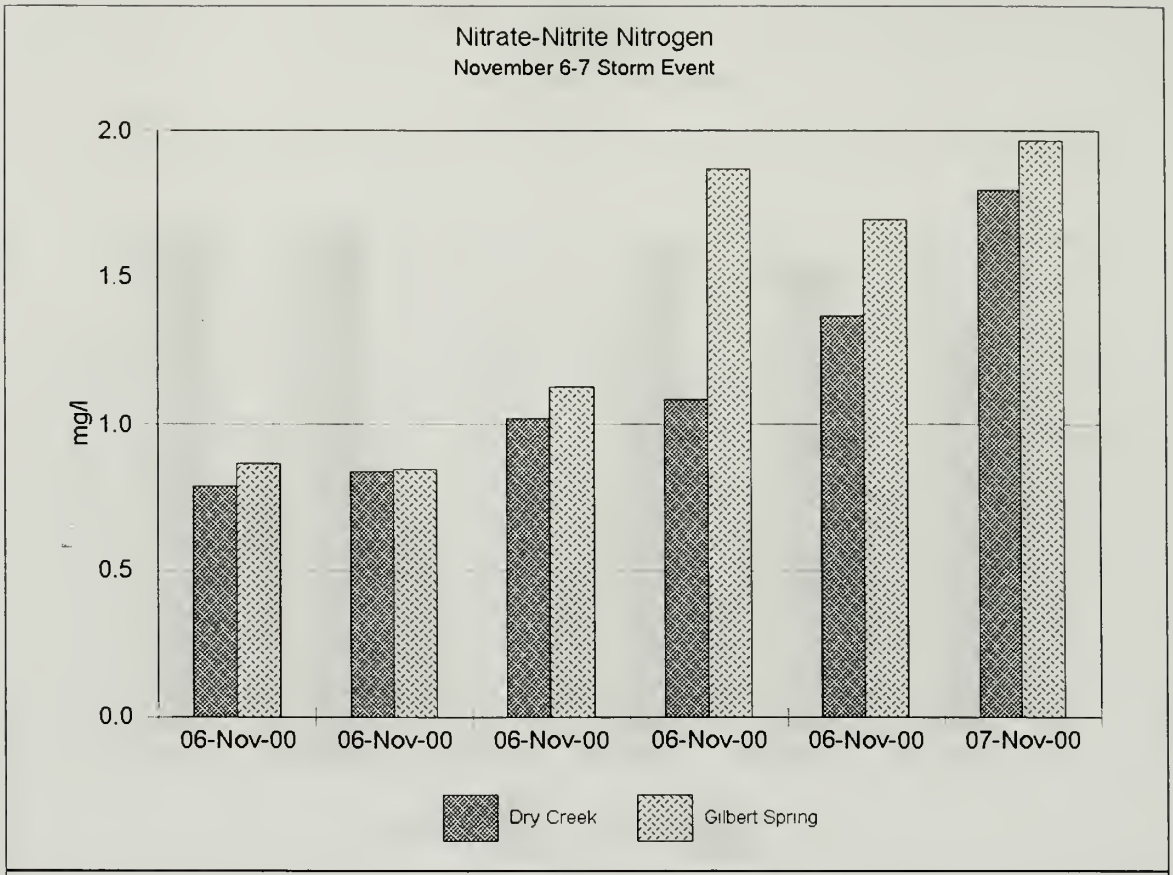
November 6-7 Storm Event

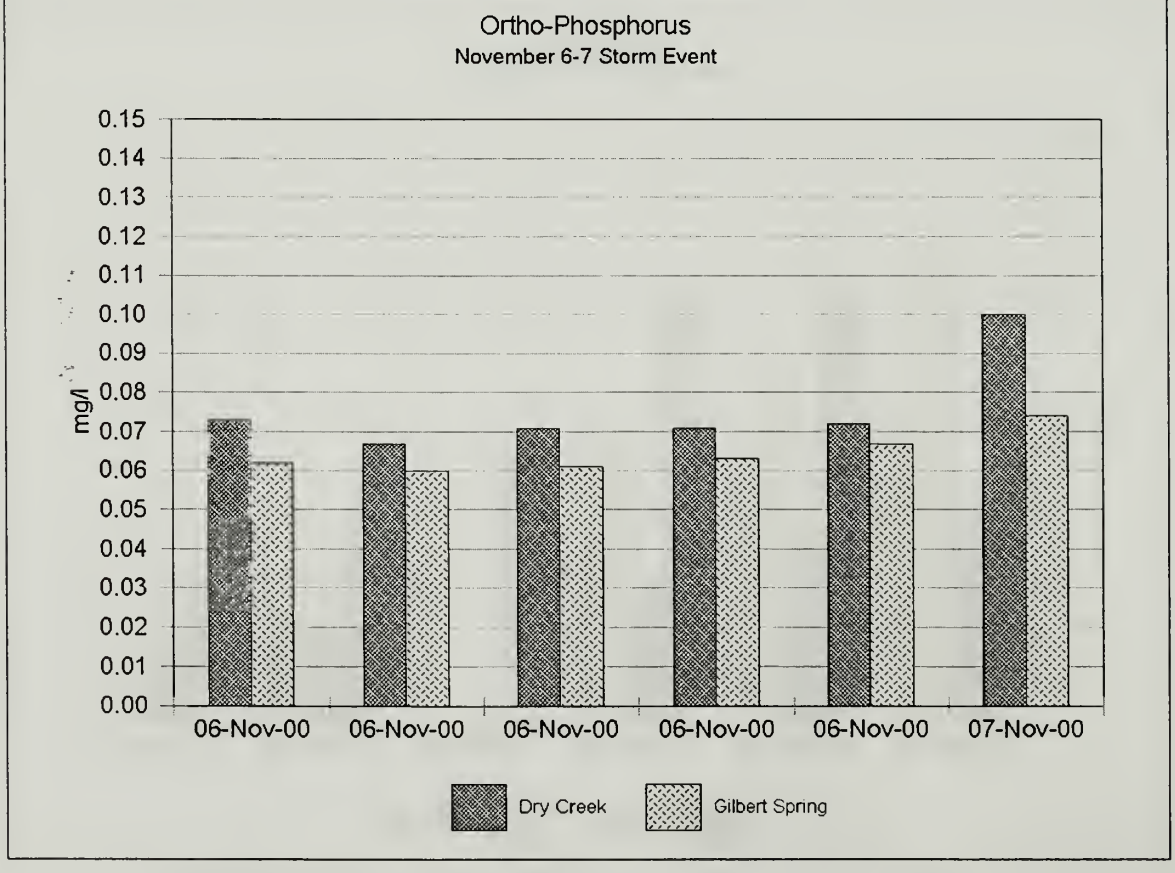


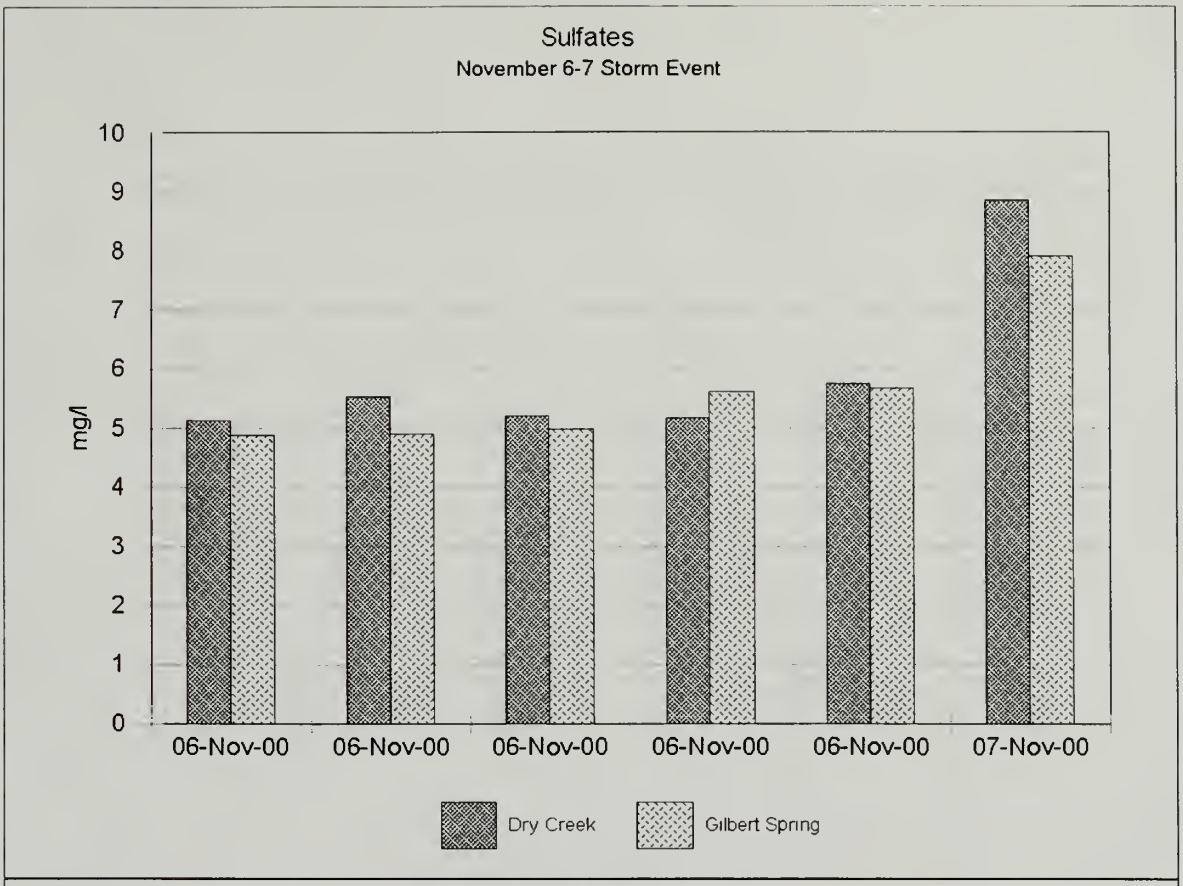
Manganese

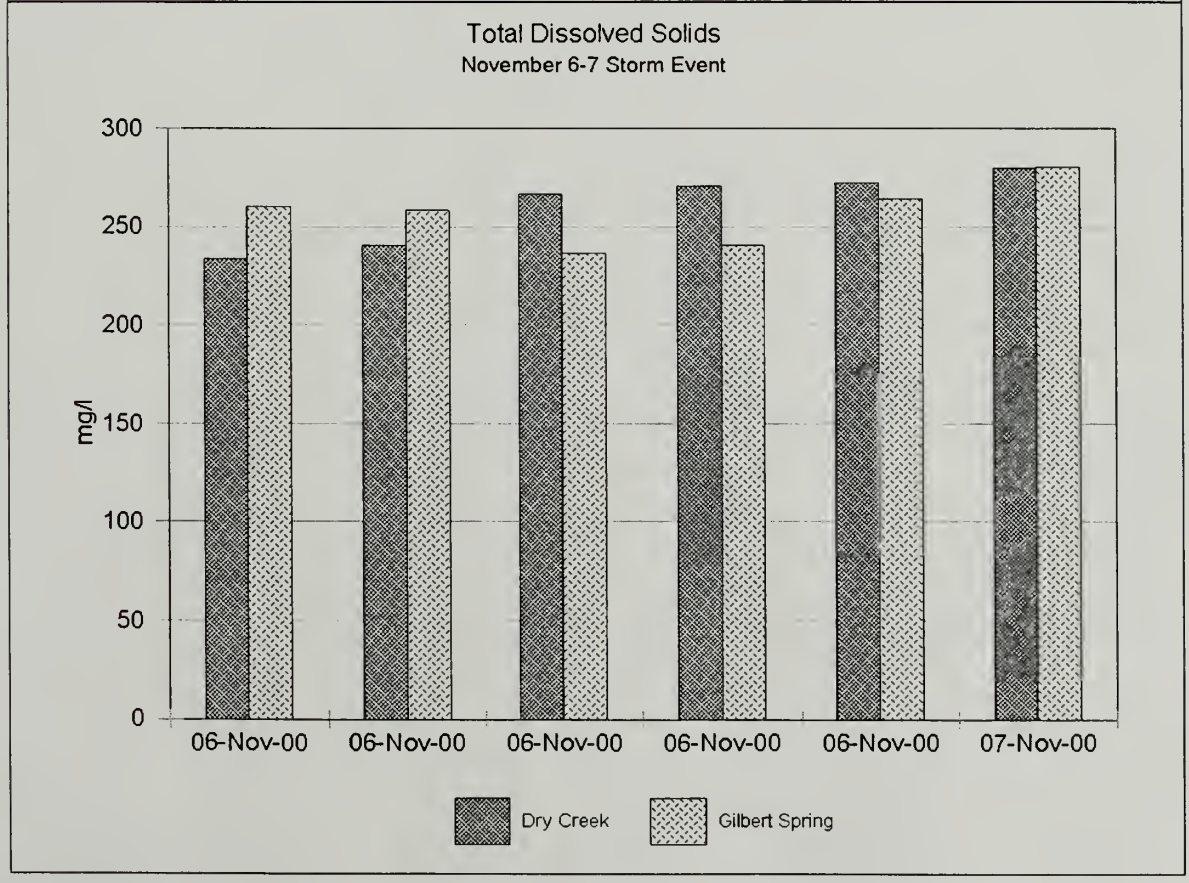
November 6-7 Storm Event




Nitrate-Nitrite Nitrogen
November 6-7 Storm Event
mg/l
2.0
1.5
1.0
0.5
0.0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

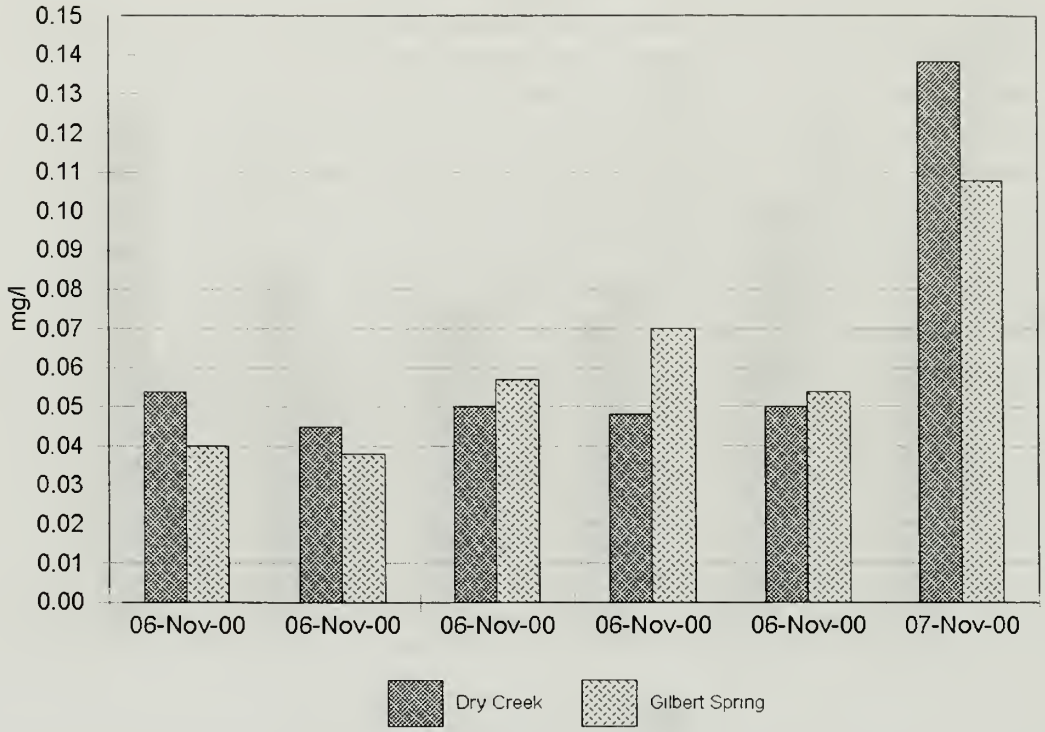

Ortho-Phosphorus
November 6-7 Storm Event
mg/l
0.15
0.14
0.13
0.12
0.11
0.10
0.09
0.08
0.07
0.06
0.05
0.04
0.03
0.02
0.01
0.00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Sulfates
November 6-7 Storm Event
mg/l
10
9
8
7
6
5
4
3
2
1
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring


Total Dissolved Solids
November 6-7 Storm Event
mg/l
300
250
200
150
100
50
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring

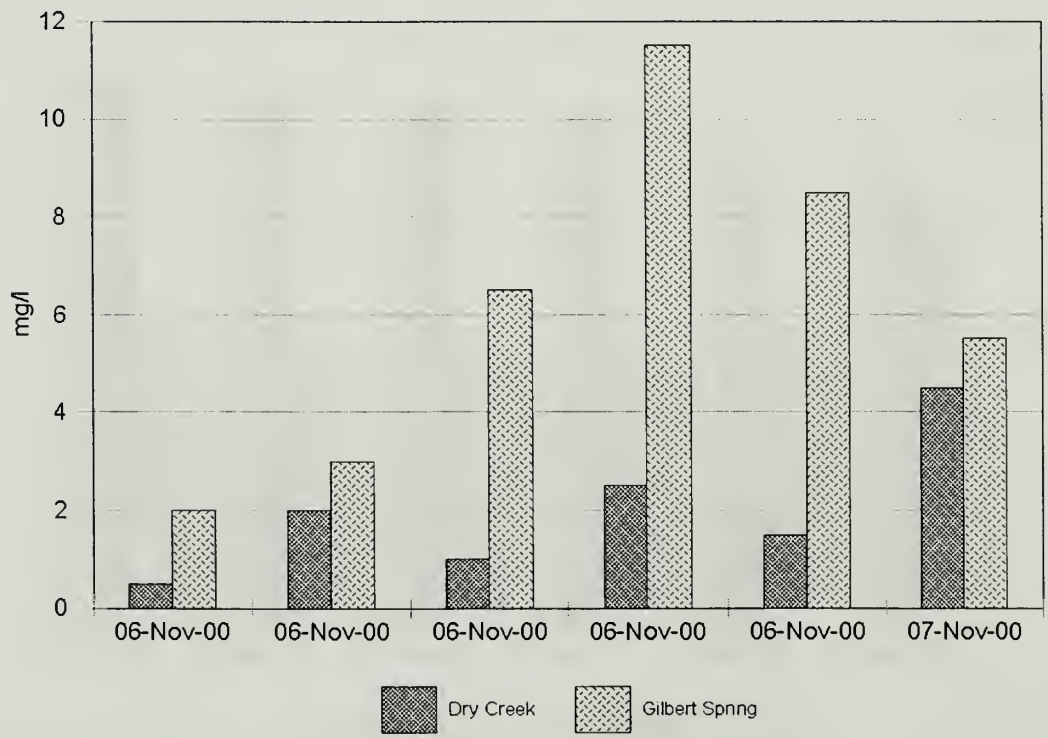
Total Phosphorus

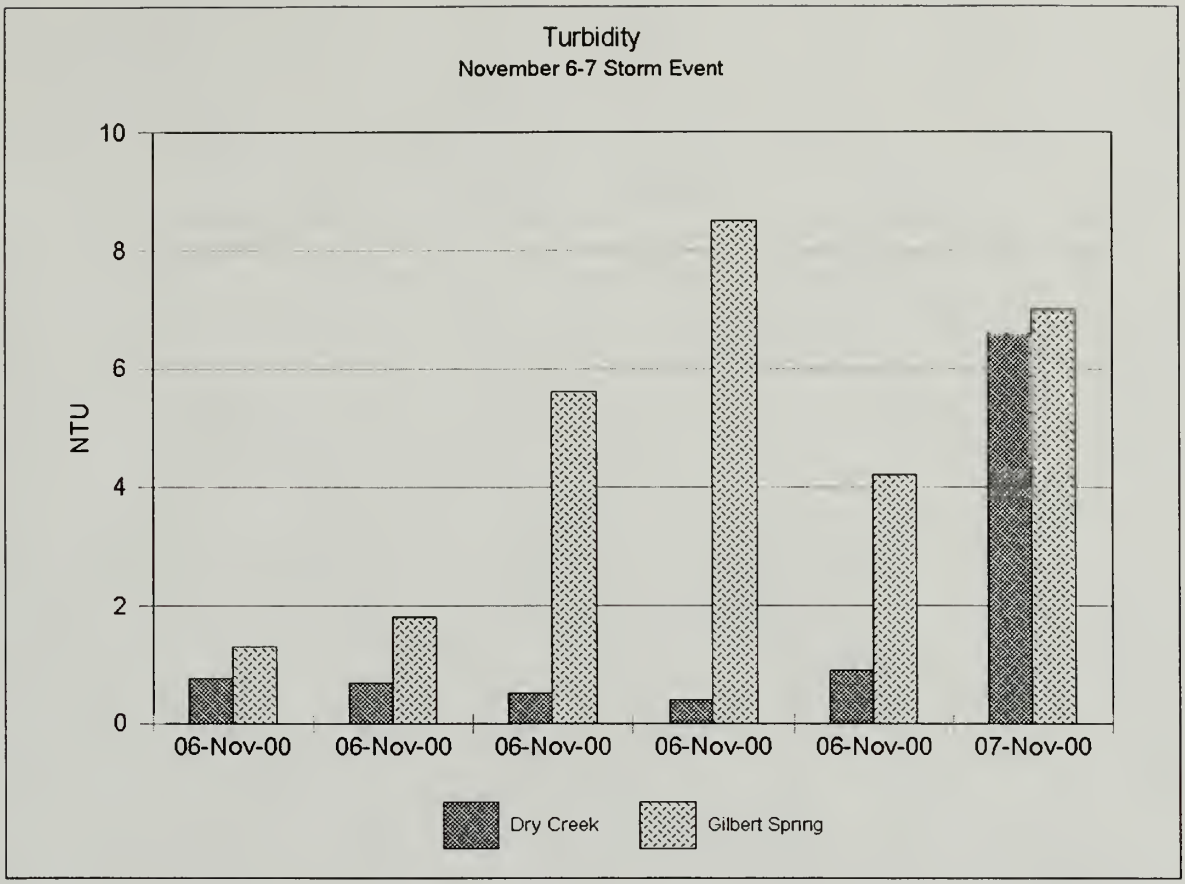
November 6-7 Storm Event



Total Suspended Solids

November 6-7 Storm Event




Turbidity
November 6-7 Storm Event
NTU
10
8
6
4
2
0
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
06-Nov-00
07-Nov-00
Dry Creek
Gilbert Spring